Mapping the neuroanatomy of ABHD16A–ABHD12 & lysophosphatidylserines provides new insights into the pathophysiology of the human neurological disorder PHARC

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Supplementary Figure (S1 – S8)

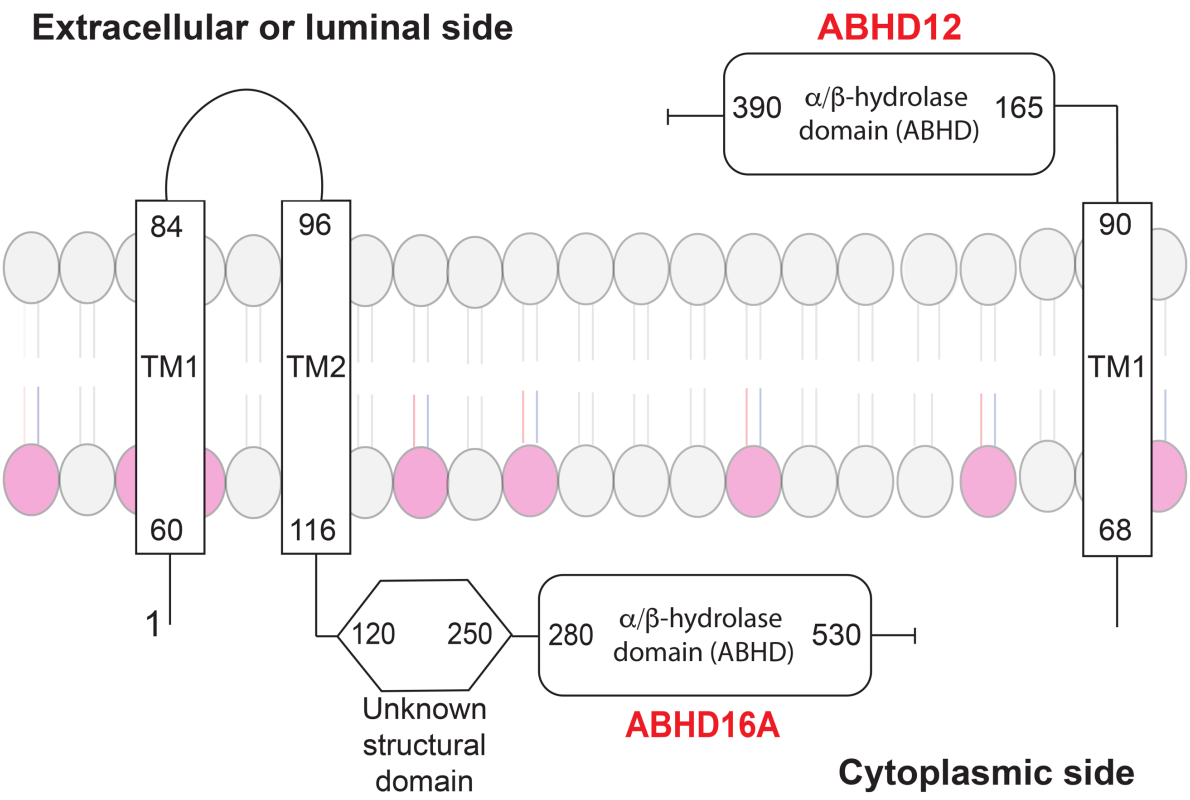

Figure S1. Schematic map of the topology of ABHD16A and ABHD12. Previous biochemical studies and computational predictions suggest that the active site of ABHD16A (α/β-hydrolase domain, amino acids 280 to 530) is cytosolically oriented, while the active site of ABHD12 (α/β-hydrolase domain, amino acids 165 to 390) is either extracellularly or luminally oriented. Further ABHD16A has two transmembrane helices (TM1, amino acids 60 to 84 and TM2, amino acids 96 to 116), while ABHD12 has a single transmembrane helix (TM1, amino acids 68 to 90). ABHD16A has an unknown domain predicted to confer structural stability to this lipase (amino acids 120 to 250).

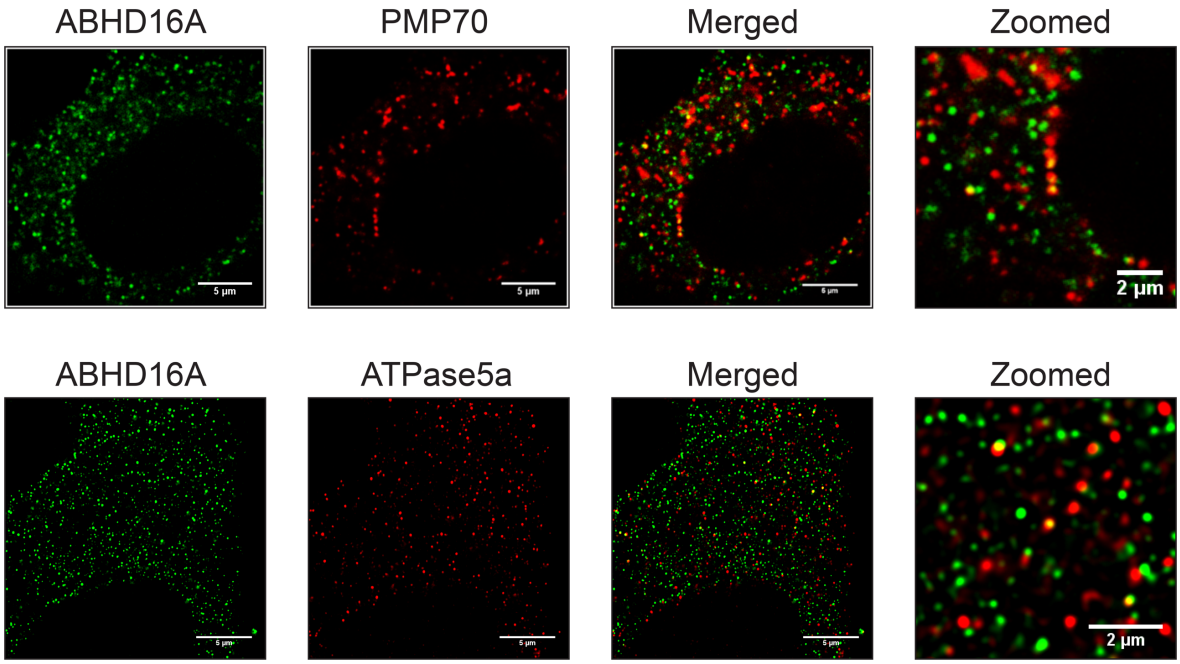

Figure S2. ABHD16A does not localize to the peroxisomes or the mitochondria. Cellular immunofluorescence studies in Neuro-2a cells showing almost no co-localization of ABHD16A with PMP70 (peroxisomal marker) or ATPase5a (mitochondrial marker). Bars on normal images are 5-μm, while the bar on the zoomed image is 2-μm. These immunofluorescence experiments were done three times, with reproducible results each time.

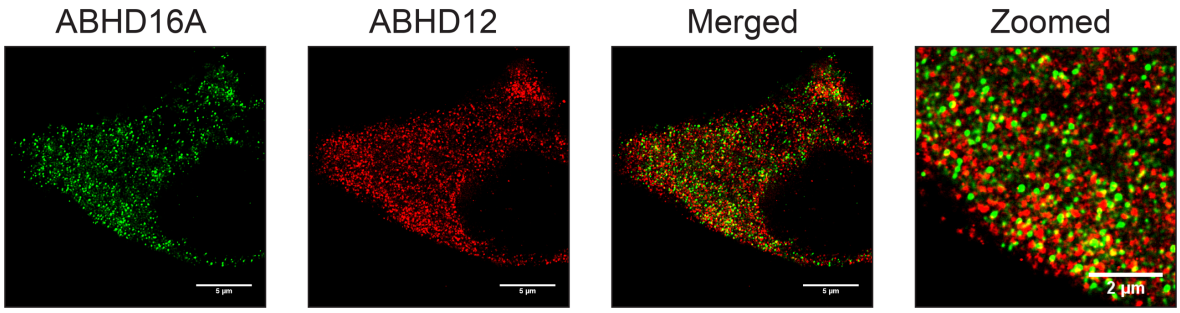

Figure S3. ABHD16A co-localizes with ABHD12. Cellular immunofluorescence studies in Neuro-2a cells showing co-localization of ABHD16A with ABHD12, further confirming its ER localization. Bars on normal images are 5-μm, while the bar on the zoomed image is 2-μm. This immunofluorescence experiment was done three times, with reproducible results each time

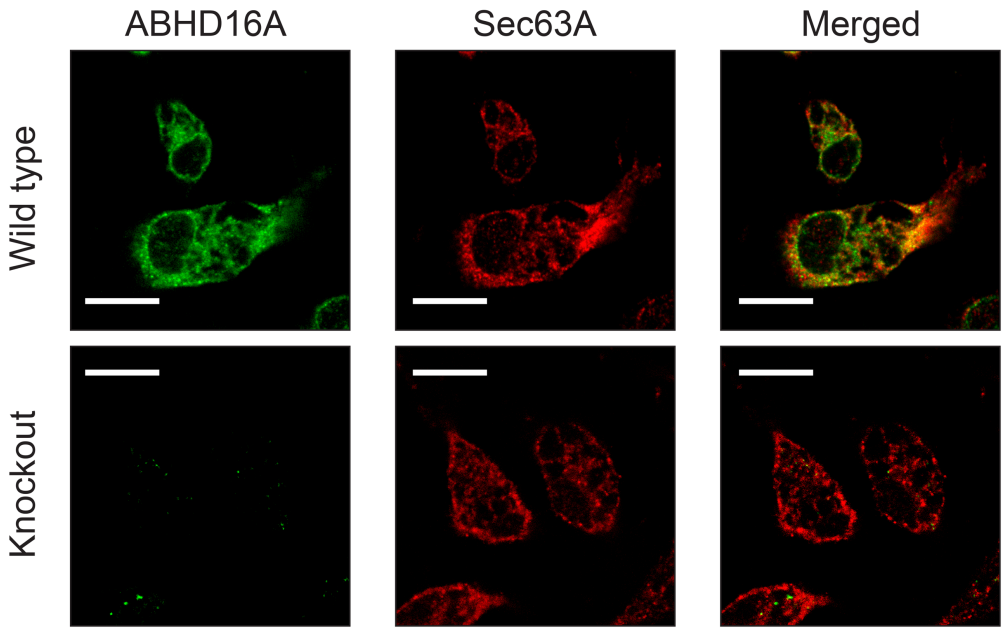

Figure S4. ABHD16A is ER localized in primary peritoneal macrophages. Cellular immunofluorescence studies on thioglycollate elicited primary peritoneal macrophage cells showing co-localization of ABHD16A with Sec63A (an ER marker), confirming its ER localization in these cells. Of note, the ABHD16A is present in cells derived from wild type mice, but not knockout mice, confirming that our immunofluorescence readout is rightly reporting on ABHD16A expression. Bars on images are 5-μm. This immunofluorescence experiment was done three times, with reproducible results each time

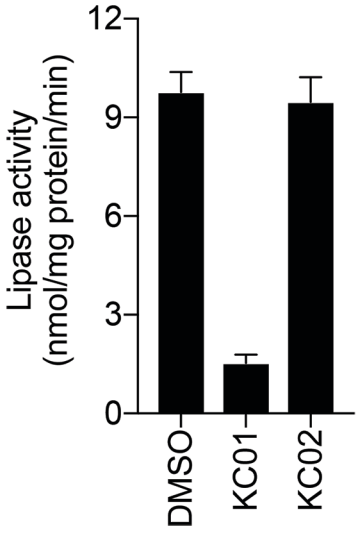

Figure S5. ABHD16A contributes majority of the cerebellar PS lipase activity. The pharmacological inhibition by KC01 (1 μM, 60 mins, 37 ^{o}C), but not KC02 (1 μM, 60 mins, 37 ^{o}C), suggests that majority (~ 90%) of the PS lipase activity in the cerebellum is contributed by ABHD16A. Data represents mean ± standard deviation for three biological replicates per group.

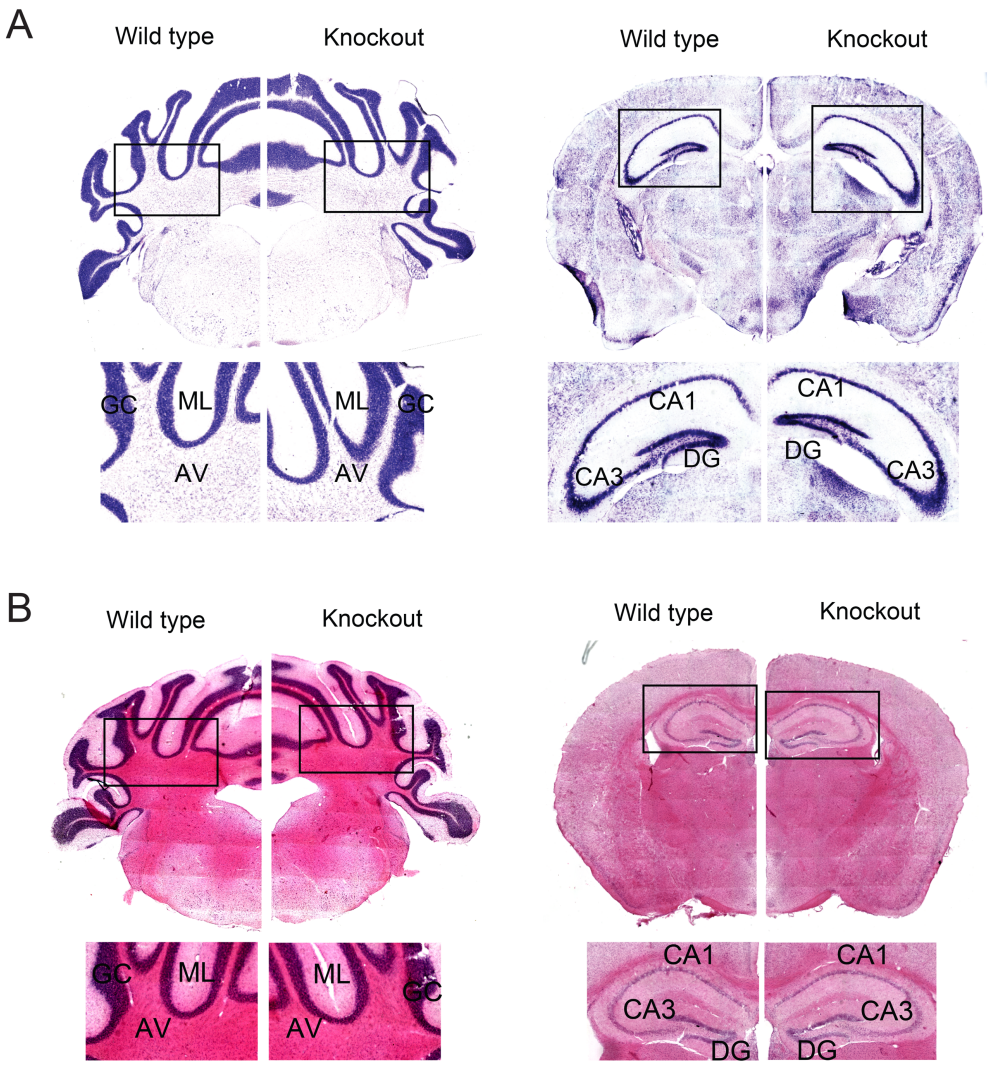

Figure S6. ABHD16A knockout mouse brains do not display any gross anatomical defects. Representative images from histochemical analysis on the coronal sections of brains obtained from wild type or ABHD16A knockout mice stained by (**A**) Cresyl Violet staining (Nissl Granules) and (**B**) Haematoxylin and Eosin (H&E) staining, showing no genotype specific gross anatomical changes. The coronal section on the left for both (**A**) and (**B**) represent a hindbrain section (cerebellum and medulla), while the right image section represents a forebrain section (hippocampus, cortex, thalamus and hypothalamus). The images below are zoomed versions of the corresponding full sectional images above them. Abbreviations in the figures are: ML – Molecular Layer, GC – Granular Layer, AV – Arbour Vitae, DG – Dentate Gyrus. These histochemical experiments were done three times, with reproducible results each time.

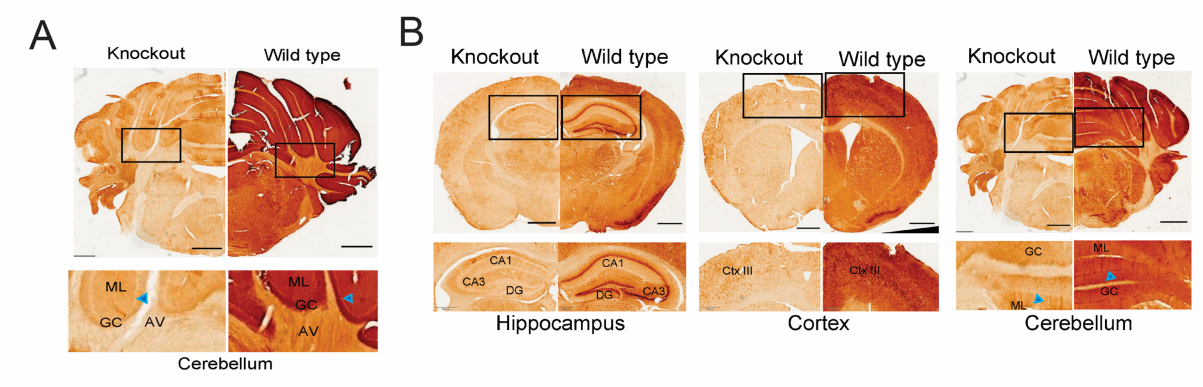

Figure S7. Mapping the anatomical distribution of ABHD16A and ABHD12 using immunohistochemical (IHC) analysis on coronal sections. Representative images from the IHC analysis on coronal brain sections from (**A**) wild type and ABHD16A knockout mice and (**B**) wild type and ABHD12 knockout mice, using DAB staining. Knockout controls for both IHC analysis show very little signal. ABHD16A is enriched in the cerebellum, while ABHD12 is present in the cortex, the hippocampus and the cerebellum. Bars on entire coronal image are 2 mm. The IHC experiments were done at least six independent times with reproducible results each time.

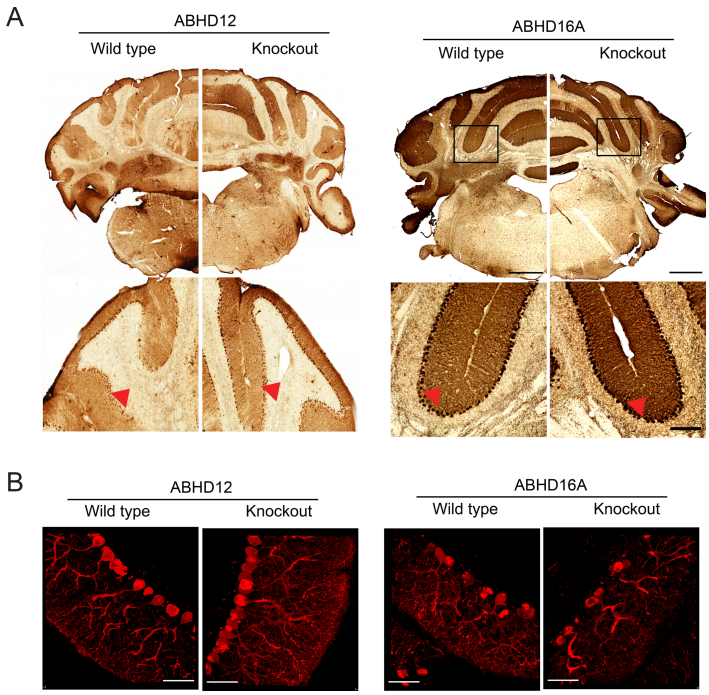

Figure S8. Purkinje neurons do not exhibit any defects in ABHD16A knockout mice or ABHD12 knockout mice. (**A**) DAB based immunohistochemical analysis (using anti-calbindin) showing similar Purkinje neurons in ganglionic layer of the cerebellum of ABHD16A knockout or ABHD12 knockout mice compared to their age and gender matched wild type littermates. (**B**) Confocal microscopy image of Purkinje neurons (anti-Calbindin) in ABHD16A knockout or ABHD12 knockout mice compared to their age and gender matched wild type littermates, showing no changes in Purkinje neuron morphology across the genotypes. These immunohistochemical experiments were done three times with reproducible results each time.