SUPPLEMENTARY INFORMATION

CPT1C negatively regulates the endocannabinoid hydrolase ABHD6 depending on nutritional status

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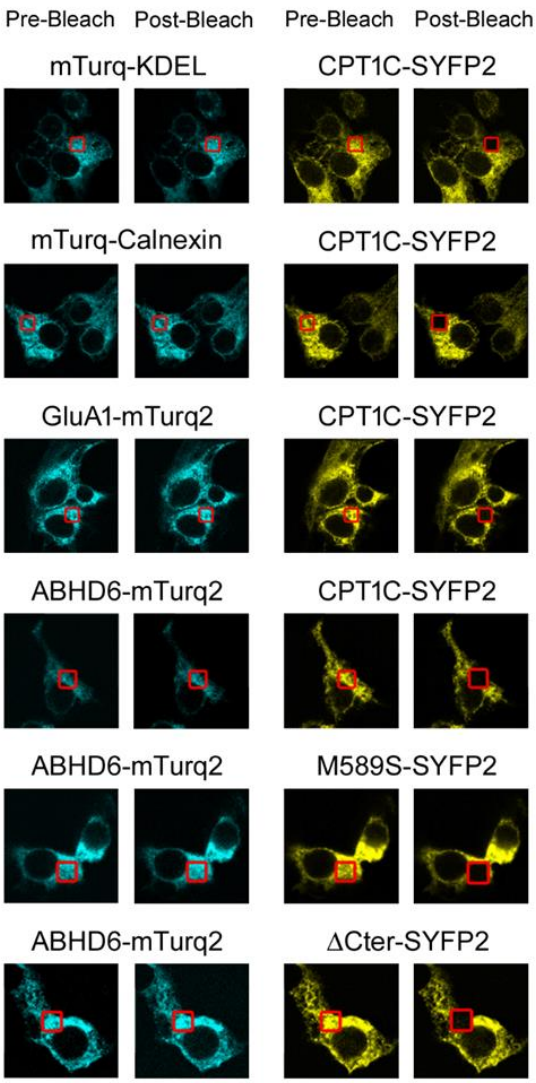
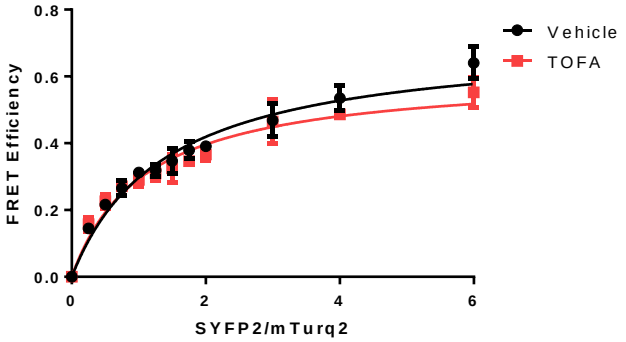
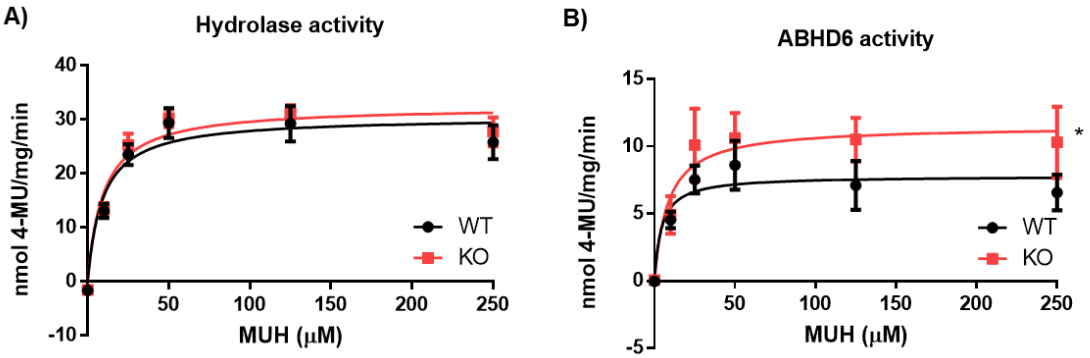


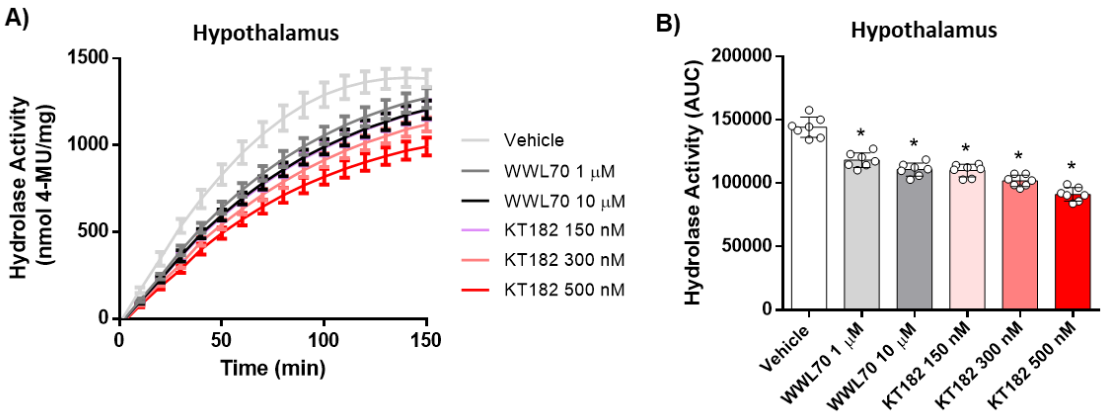
Supplementary Figure S1. Representative FRET images of transfected HEK-293T cells with proteins fused to mTurquoise2 (donor; blue) or SYFP2 (acceptor; yellow) before and after bleaching.



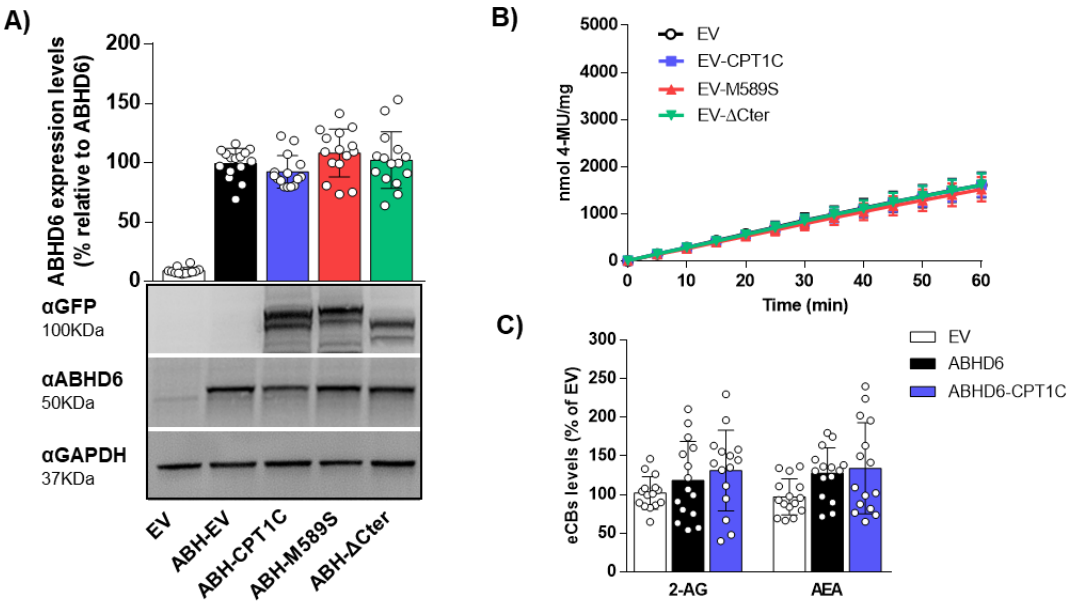
Supplementary Figure S2. FRET sensitized emission of CPT1C with ABHD6 in the presence of an ACC inhibitor in living cells. Assays were performed 48h post-transfection in HEK-293T cells expressing ABHD6-mTurq2 and increasing amounts of the cDNA for CPT1C-SYFP2, in the presence or absence of the ACC inhibitor, TOFA (20 µg/ml, 2h). FRET saturation curves were obtained by monitoring the SYFP2 fluorescence emission at 530 nm after excitation of mTurq2 at 420 nm, with subtraction of the values obtained with cells expressing the same amount of donor protein. Mean±SD (n=4).



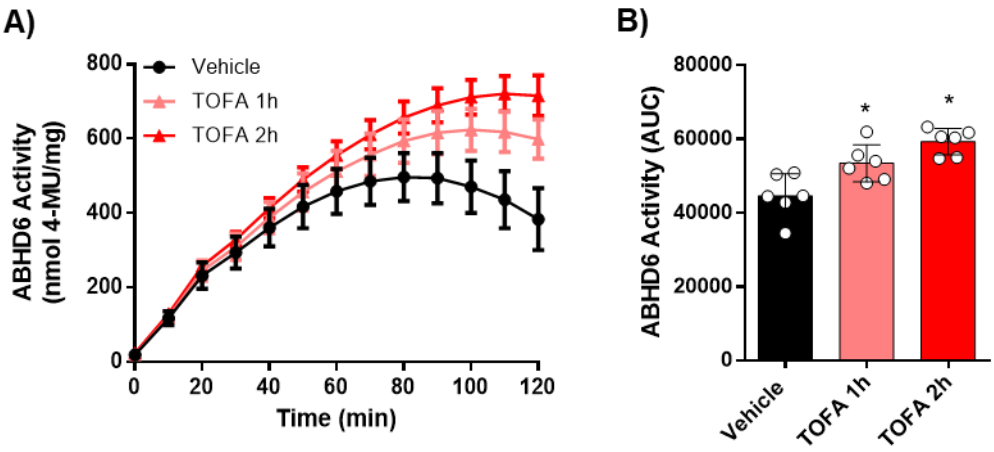
Supplementary Figure S3. ABHD6 assay based on 4-MUH hydrolysis in hypothalamus of WT and CPT1C-KO mice. ABHD6 activity dependent on 4-MUH concentration at t=30 min in the absence (A) or presence of 10 µM WWL70 (B). Two-way ANOVA followed by Bonferroni's post-hoc correction was used for statistical analysis. Mean±SD (n=6). *P<0.05 versus WT.



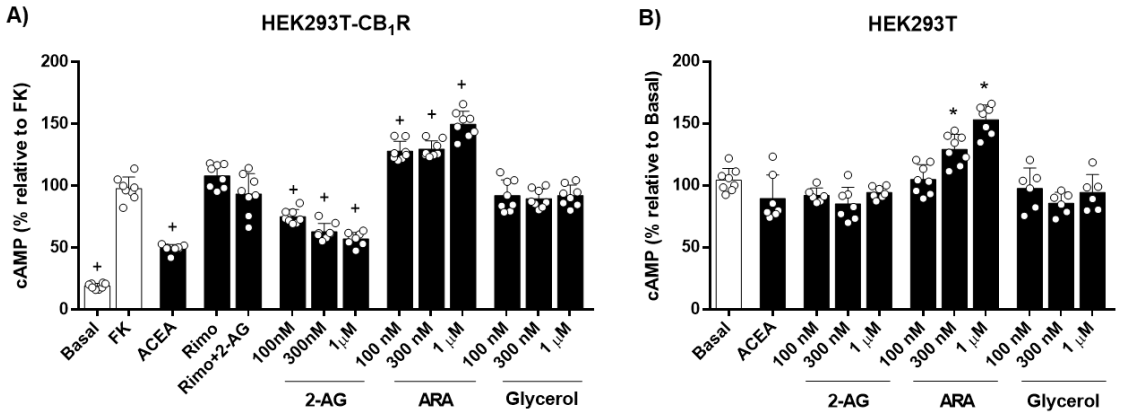
Supplementary Figure S4. Kinetic hydrolase activity (A) and areas under the curve (B) in the absence or presence of the ABHD6 inhibitors WWL70 and KT182. Mean±SD (n=7). *$P<0.05$ versus Vehicle.



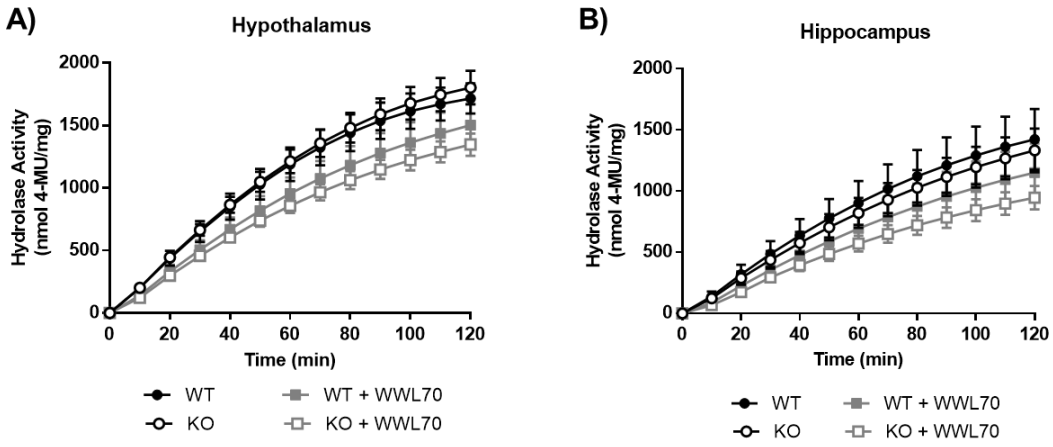
Supplementary Figure S5. ABHD6 activity assay based on 4-MUH hydrolysis. (A) Blotting and protein expression levels of ABHD6 in combination with CPT1C, M589S or ΔCter in HEK-293T for ABHD6 activity assays (n=15). (B) 4-MUH hydrolysis of HEK-293T cells expressing EV, CPT1C, M589S and Δcter (n=4). (C) HEK-293T endogenous endocannabinoids (eCBs) levels, 2-AG and AEA, when transfected with EV, ABHD6 or ABHD6-CPT1C (n=15). Mean±SD.



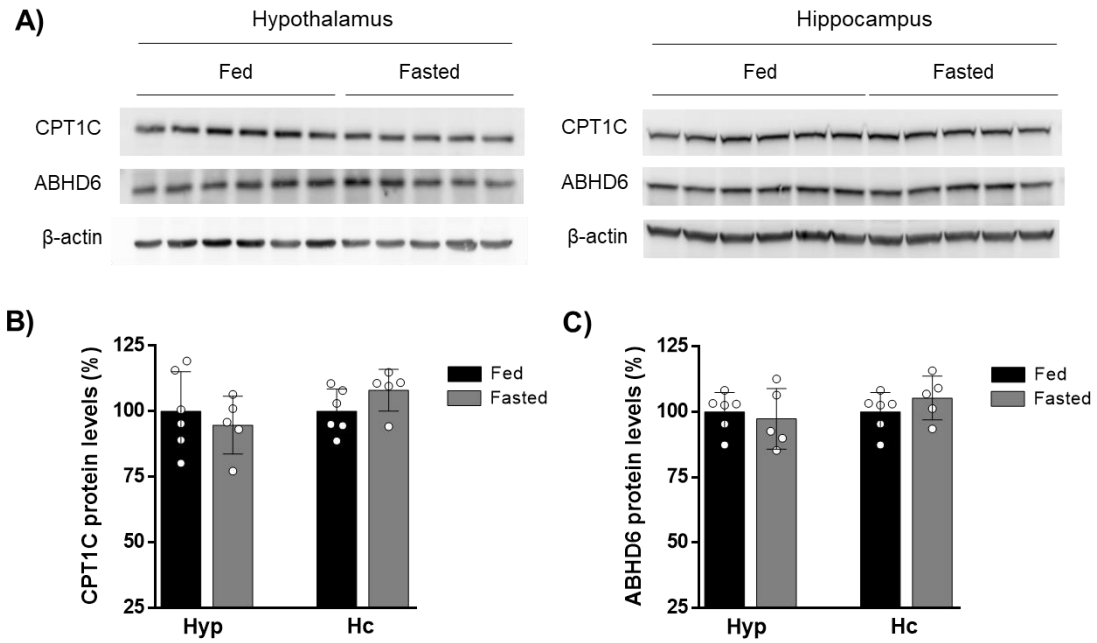
Supplementary Figure S6. ABHD6 activity assay based on 4-MUH hydrolysis in a neuronal cell line, GT1-7, endogenously expressing ABHD6 and CPT1C. Time course ABHD6 activity assay (A) and areas under the curve (B) in GT1-7 cells with or without TOFA treatment (20 µg/mL) for 1h and 2h. Mean±SD (n=6). *P<0.05 versus vehicle.



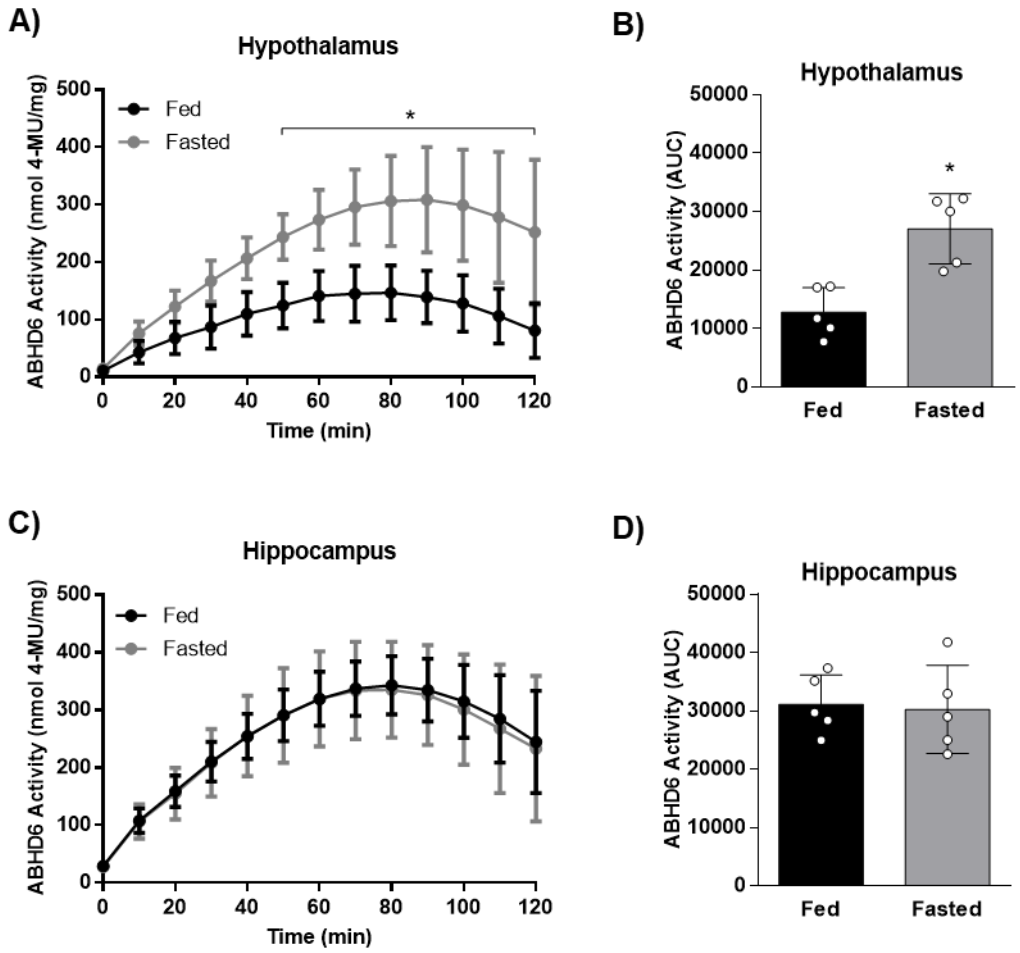
Supplementary Figure S7. Intracellular cAMP assay was set up in HEK-293T. (A) Forskolin (FK)-induced cAMP levels of HEK-293T expressing CB_1R incubated with an agonist of CB_1R, ACEA, an antagonist, rimonabant (Rimo), Rimo + 2-AG and increasing concentrations of 2-AG, arachidonic acid (ARA) and glycerol. (B) Effect of ACEA, 2-AG, ARA and glycerol in basal cAMP levels of HEK-293T cells alone. Mean±SD (n=8). $^{+}P<0.05$ versus FK and $^{*}P<0.05$ versus basal.



Supplementary Figure S8. ABHD6 assay based on 4-MUH hydrolysis in brain tissue homogenates of WT or CPT1C-KO male mice in the absence or presence of the ABHD6 inhibitor, WWL70 (10 µM). (A) 4-MUH hydrolysis in hypothalamic homogenates. (B) 4-MUH hydrolysis in hippocampal homogenates. Mean±SD (n=6).



Supplementary Figure S9. CPT1C and ABHD6 protein expression on brain tissue homogenates of fed or fasted mice. Representative blots (A) and quantification (% relative to WT-fed) of CPT1C (B) and ABHD6 (C) protein levels in either hypothalamus (Hyp) or hippocampus (Hc) of WT mice under fed (n=6) or fasting conditions (n=5). Mean±SD.



Supplementary Figure S10. Effect of fasting on ABHD6 activity of female WT brain tissues. (A, B) ABHD6 activity in hypothalamic homogenates and area under the curves of mice after fed or fasted state. (C, D) ABHD6 activity in hippocampal homogenates and area under the curves of mice after fed or fasted state. Mean±SD (n=5). *P<0.05 versus WT-fed.