

Supplementary materials accompanying

**Identification of α,β -hydrolase domain containing protein 6
as a diacylglycerol lipase in Neuro-2a cells**

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1 Supplementary Figures

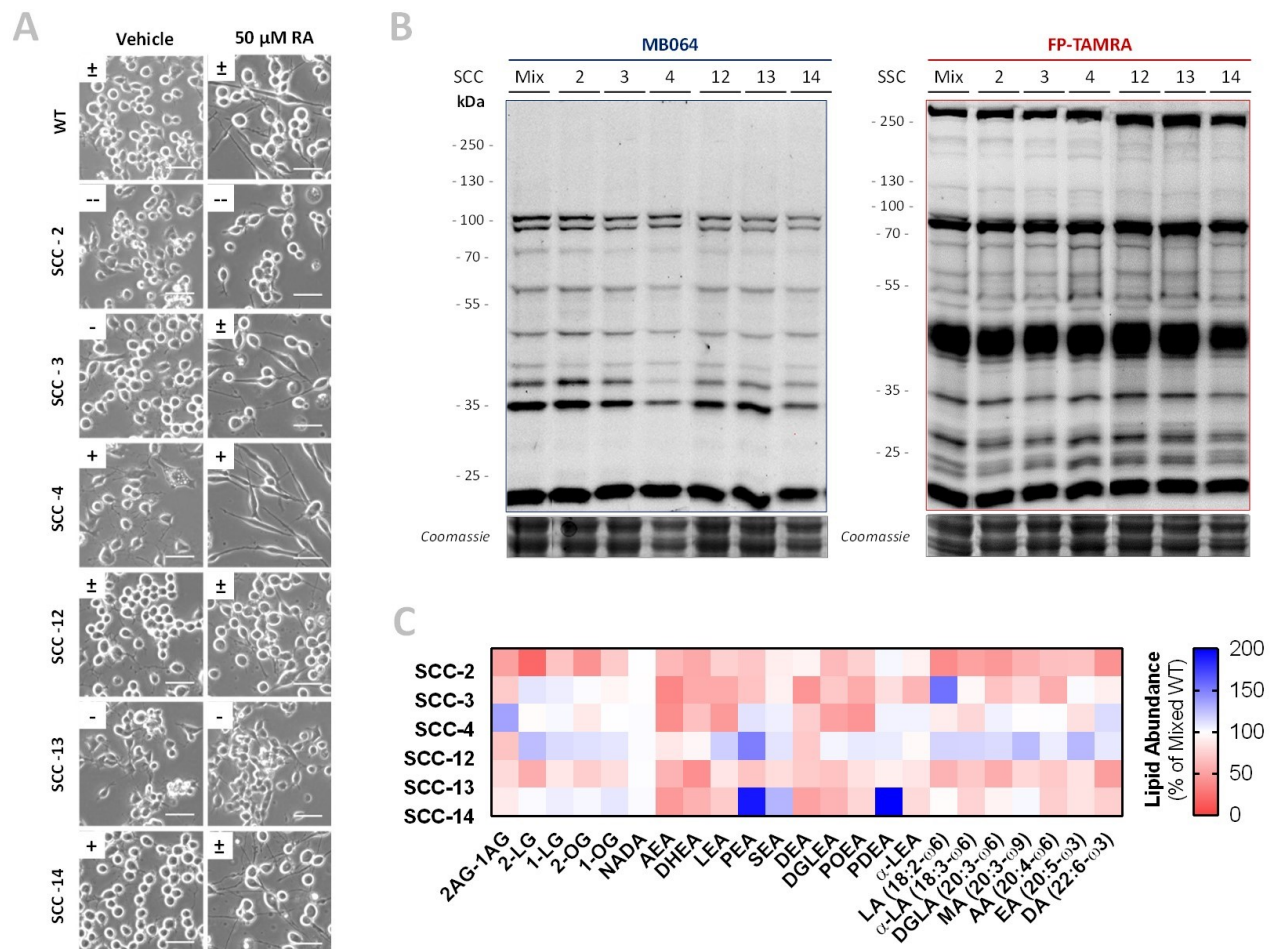


Figure S1 | Neuro-2a single cell clones have diverging phenotypes and lipid profiles.

(A) Neuro-2a and Neuro-2a single cell clones (SCCs) show a diverging extent of neurite outgrowth upon treatment with retinoic acid (50 μ M, 0.1% DMSO, 2% serum, 48 hrs.). -/+ indicate the state of neurite outgrowth as compared to the mixed culture under the same conditions. Scale bar: 50 μ m. (B) WT Neuro-2a and six different WT Neuro-2a SCCs were analyzed by gel-based ABPP using probes MB064 (250 nM) and FP-TAMRA (500 nM) (20 min, rt). Coomassie served as a protein loading control. (C) Heat map summary of lipidomics performed on Neuro-2a and Neuro-2a SCCs revealed variation in lipid abundance between clones. Lipid abundance was normalized to cell number and data is expressed as % of WT (n=3).

Supplementary Materials: Identification of ABHD6 as a diacylglycerol lipase in Neuro-2a cells

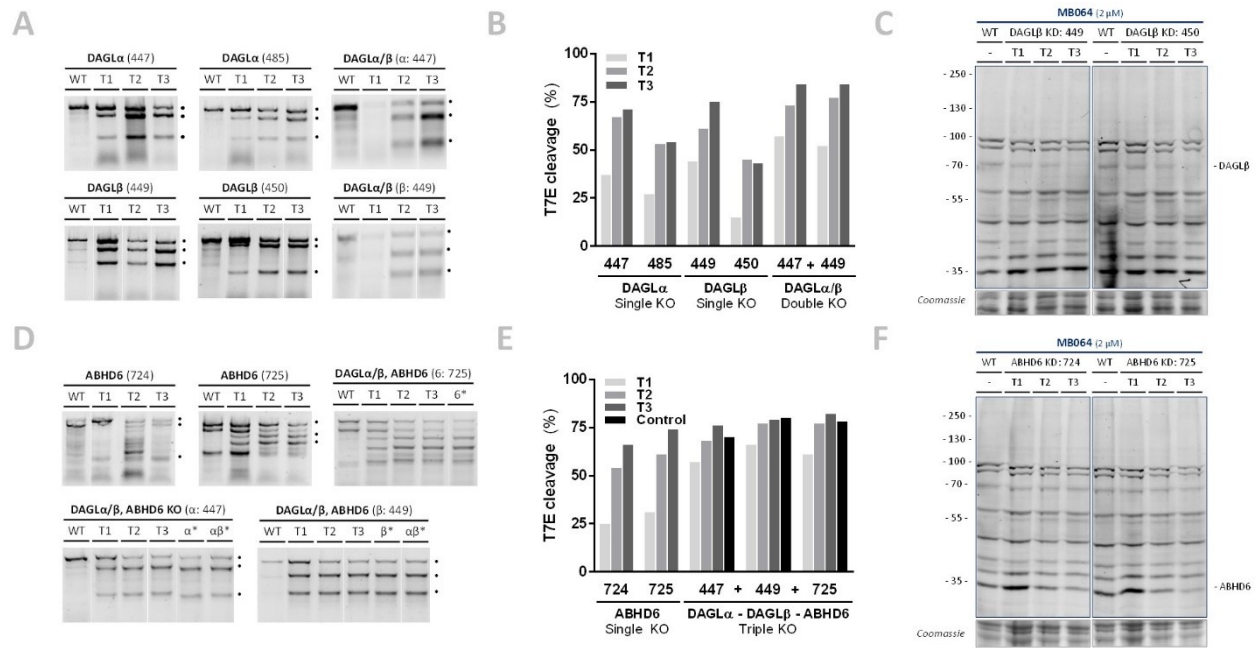


Figure S2 | Efficiency of CRISPR/Cas9-mediated knockdown in Neuro-2a. Knockdown populations were generated by three sequential transfections (T1-T3) with Cas9 and two different separate guides for each target. Most efficient guides were used to generate double and triple knockdowns. **(A-B, D-E)** Knockdown efficiency is determined by a T7E1 assay on genomic DNA after each round of transfection and quantified for DAGL **(A-B)** and ABHD6 **(D-E)** knockdowns. **(C, F)** ABPP analysis of knockdown efficiency. After each transfection, whole lysates were incubated with MB064 (2 μ M, 20 min, rt) and analyzed by SDS-PAGE.

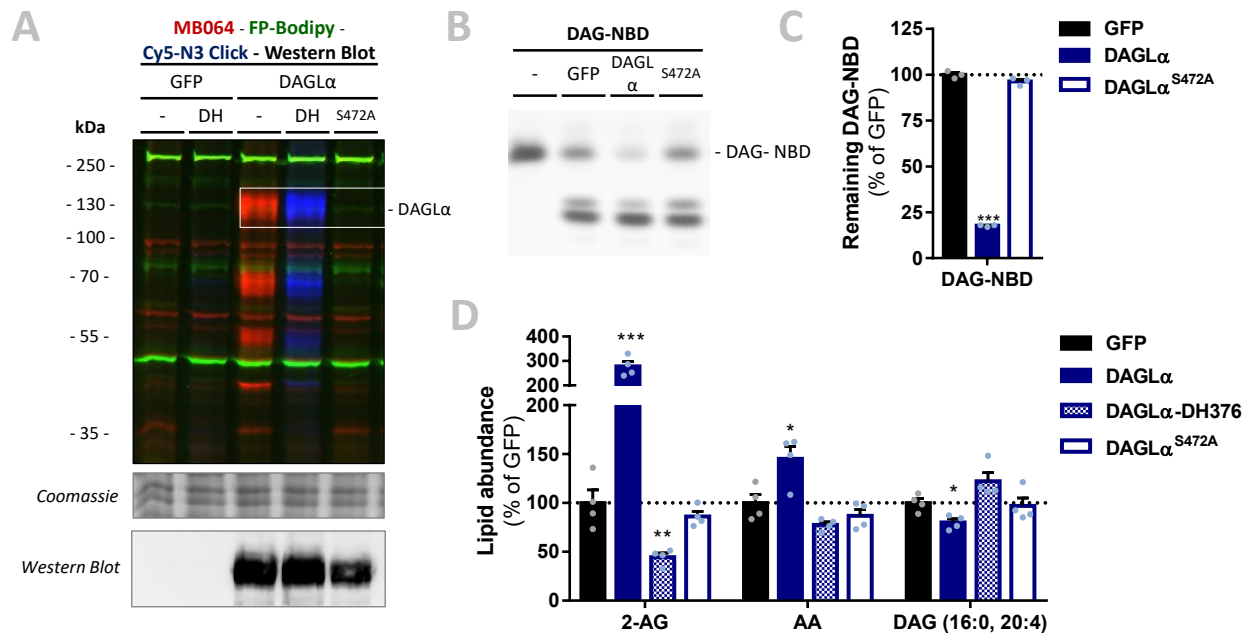


Figure S3 | Recombinant DAGLα possesses DAG-lipase activity *in vitro* and *in situ*. HEK293-T cells were transiently transfected with GFP, DAGLα or its catalytically inactive serine mutant (S472A) and treated *in situ* with vehicle or DH376 (DH, 1 μM, 2 hrs., serum-free). (A) Protein activity and expression were confirmed by gel-based ABPP and western blot. Samples were subsequently incubated with probes MB064 (500 nM, 10 min, rt), FP-BODIPY (500 nM, 10 min, rt), and Cy5-azide click mix (2.5 μM, 30 min, rt). Coomassie served as a protein loading control. Western blot with mouse-anti-FLAG (1:2500, 45 min, rt) verified expression of the catalytically inactive protein. (B-C) Whole lysates were incubated with DAG-NBD (5 μM, 30 min, 37 °C). Lipids were extracted and analyzed by HPTLC (n=3). DAG hydrolysis was quantified and expressed as % of GFP (mean ± SEM (n=3), *t*-test: *** *p* < 0.001). (D) Lipid abundance of transfected and *in situ* treated cells was measured and normalized to the amount of protein (n=4). Data is expressed as % of GFP-Vehicle (mean ± SEM (n=4), *t*-test: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001).

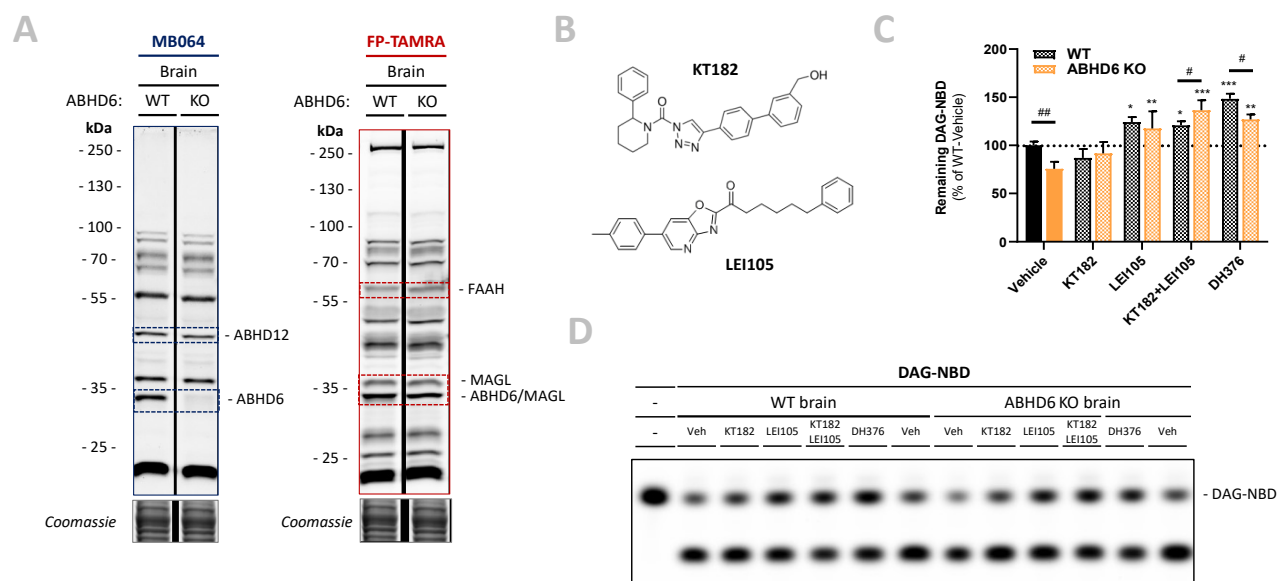


Figure S4 | DAG-NBD hydrolysis in mouse brain homogenates is not affected by KO or inhibition of ABHD6 alone. Mouse brain homogenates were prepared from mice with a WT or *Abhd6*^{-/-} background. (A) ABHD6 KO was verified by gel-based ABPP. Mouse brain homogenates were incubated with probe MB064 (250 nM, 20 min, rt) or FP-TAMRA (500 nM, 20 min, rt). Coomassie served as a protein loading control. (B-D) Brain homogenates were incubated with vehicle or inhibitors of ABHD6 (KT182, (B)), DAGL (LEI105 (B)) or dual DAGL-ABHD6 inhibitor DH376 (100 nM 30 min, 37 °C) prior to incubation with DAG-NBD (10 μM, 15 min, 37 °C). (D) Lipids were extracted and analyzed by HPTLC. (C) DAG hydrolysis was quantified and expressed as % of WT-Vehicle (mean ± SEM (n=3), *t*-test Inhibitor versus Vehicle: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001; WT versus KO: # *p* < 0.05, ## *p* < 0.01).

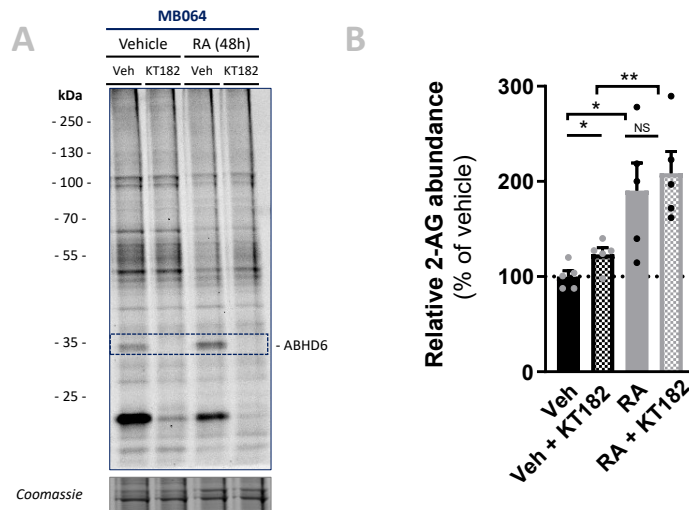


Figure S5 | ABHD6 inhibitor KT182 does not abolish increased 2-AG levels upon RA-induced differentiation. Neuro-2a cells were stimulated by *in situ* treatment with retinoic acid (RA, 50 μ M, 2% serum, 48 h) with or without co-treatment with ABHD6 inhibitor KT182 (100 nM). **(A)** Whole lysates of vehicle or RA stimulated cells were analyzed by gel-based ABPP using activity-based probe MB064 (2 μ M, 20 min, rt). Coomassie served as a protein loading control. **(B)** Lipidomics analysis on (co-)treated Neuro-2a (48 hrs). Lipid abundance was normalized to protein concentration. Data is expressed as % of vehicle (mean $\pm$ SEM (n=5), *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS not significant).

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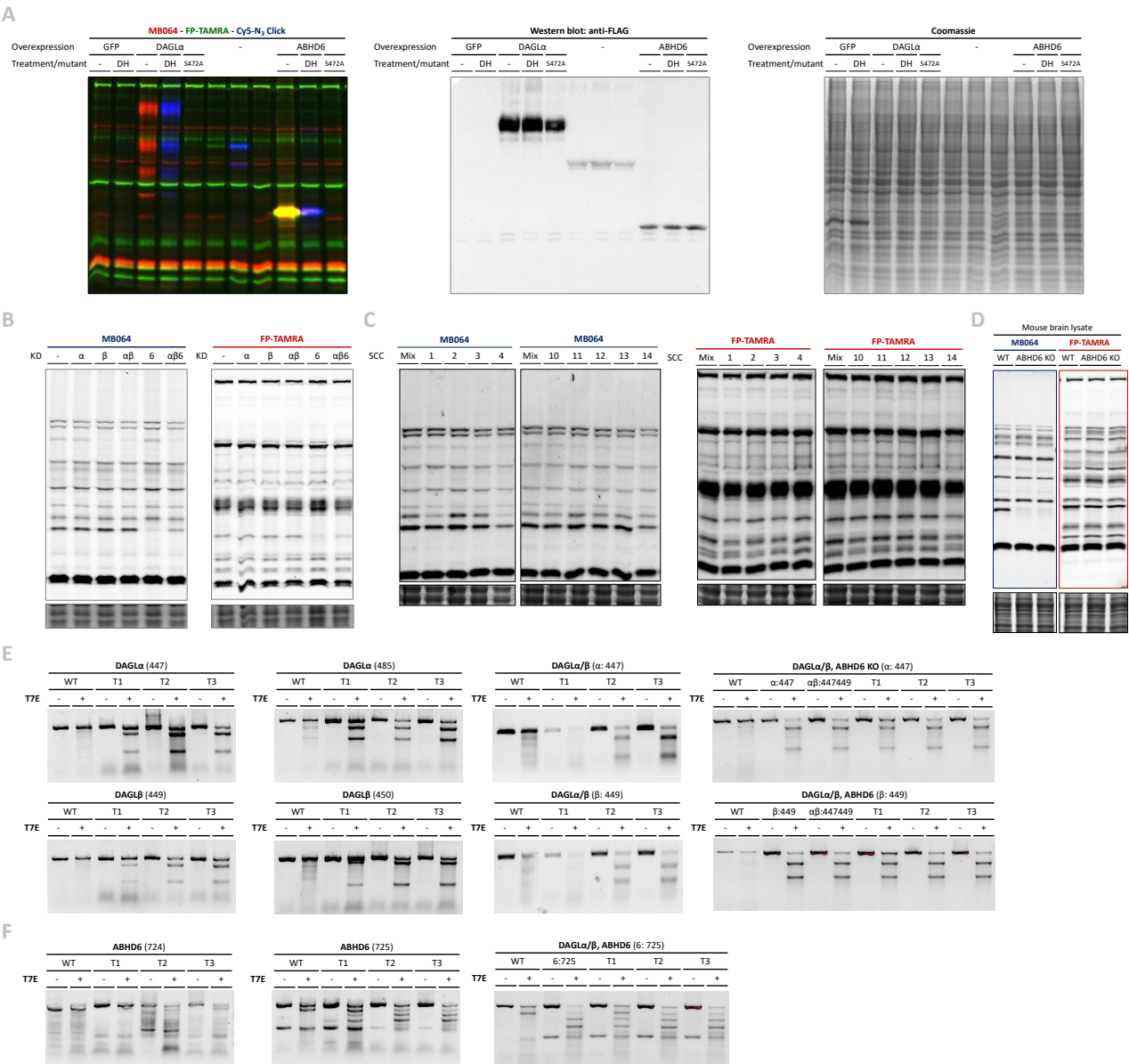


Figure S6 | Full images of cropped/combined gels and blots. Experimental details are described in the original figure captions, relevant lanes are annotated. (A) Figure 5A; (B) Figure 6A; (C) Figure S1B; (D) Figure S4A; (E) Figure S2A, D; (F) Figure S2D.

2 Supplementary files

Supplementary Table 1 - Peptide Lists (Esbroeck et al., Frontiers, 2019).xlsx

Supplementary Table 1 | Peptide lists of LC-MS based chemical proteomics / activity-based proteomics experiments.