SUPPLEMENTAL FIGURES

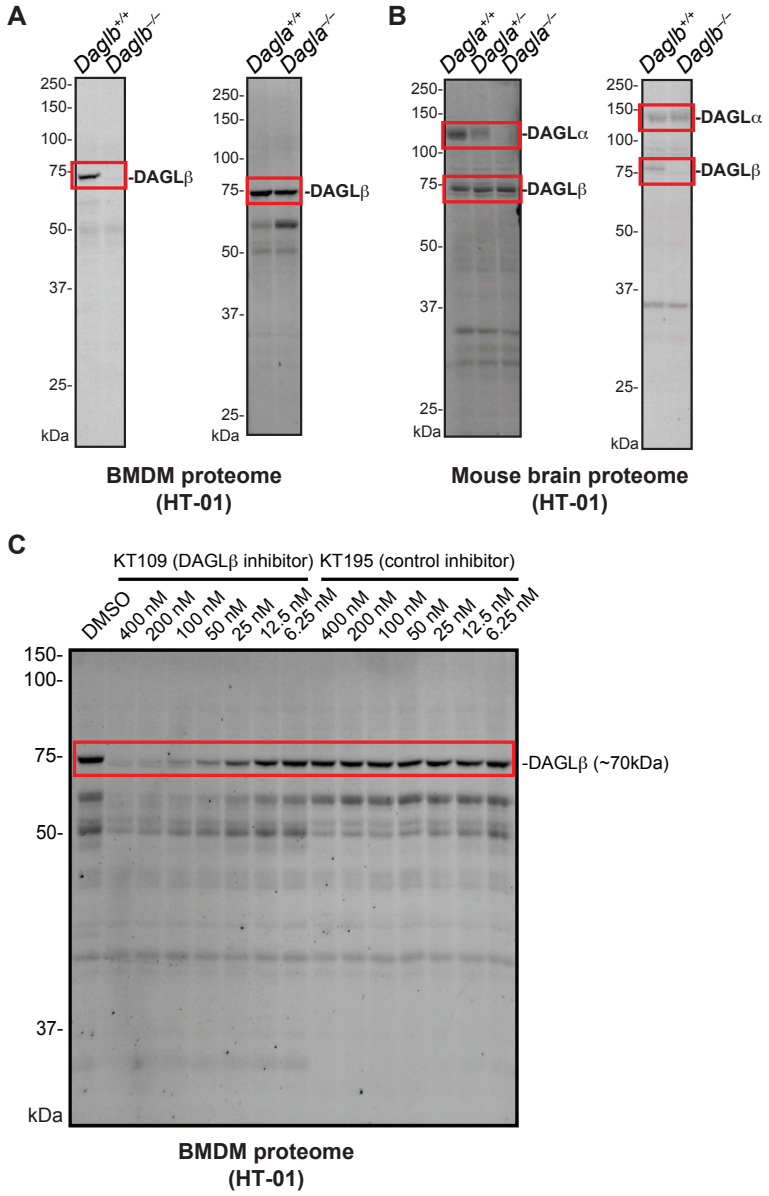

Figure S1. **Related to Figures 1 and 3. HT-01-based activity-based protein profiling (ABPP) of DAGL activity in primary macrophage and brain proteomes**. (A) Native DAGLβ but not DAGLα activity was detected in BMDMs as judged by gel-based ABPP analyses with HT-01. BMDM membrane proteomes were treated with the DAGL-tailored ABPP probe HT-01 (1 μM, 30 min, 37 °C) followed by SDS-PAGE and in-gel fluorescence scanning to measure active DAGLs in BMDM proteomes. (B) Native DAGLα and DAGLβ were active and detected in mouse brain proteomes. Gel-based ABPP analysis of brain membrane proteomes from DAGL wild-type (*Dagl*$^{+/+}$), heterozygous (*Dagl*$^{+/-}$), and knockout (*Dagl*$^{-/-}$) mice treated with HT-01 probe. (C) BMDMs were treated with varying concentrations of the DAGLβ inhibitor (KT109) or matching negative control compound (KT195) for 4 hrs at 37 °C followed by cell lysis and HT-01-based ABPP profiling of membrane proteomes (1 μM probe, 30 min, 37 °C). KT109 but not KT195 showed concentration-dependent blockade of HT-01 labeling of endogenous DAGLβ in proteomes from compound-treated cells.

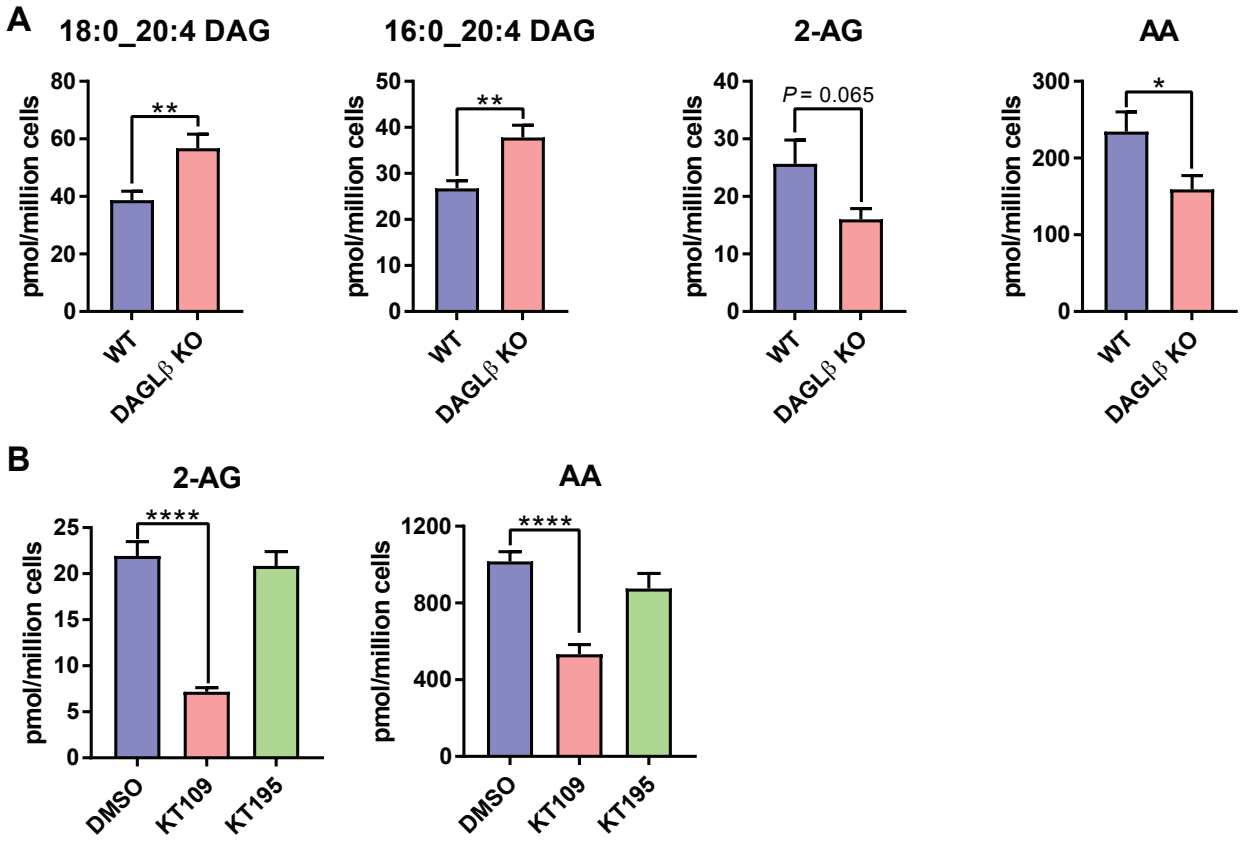

Figure S2. **Related to Figures 1 and 3. Metabolomics analysis of DAGLβ-disrupted BMDMs**. (A) Targeted metabolomics demonstrated that genetic knockout of DAGLβ resulted in cellular accumulation of lipid substrates (DAG) and depletion of products (2-AG, arachidonic acid or AA) in BMDMs. Statistical significance was determined using a two-tailed t-test (*$P < 0.05$, **$P < 0.01$; n=5-10 biological samples). (B) Targeted metabolomics showed that pharmacological blockade of DAGLβ reduced cellular 2-AG and AA in wild-type BMDMs as expected based on the role of DAGLβ in endocannabinoid biosynthesis (n=6-11 biological samples). Statistical significance was determined using a one-way ANOVA test with Dunnett multiple comparison correction (****$P < 0.0001$). All data shown represent mean ± s.e.m. and are representative of one or two independent experiments (n=1-2 biologically independent experiments).

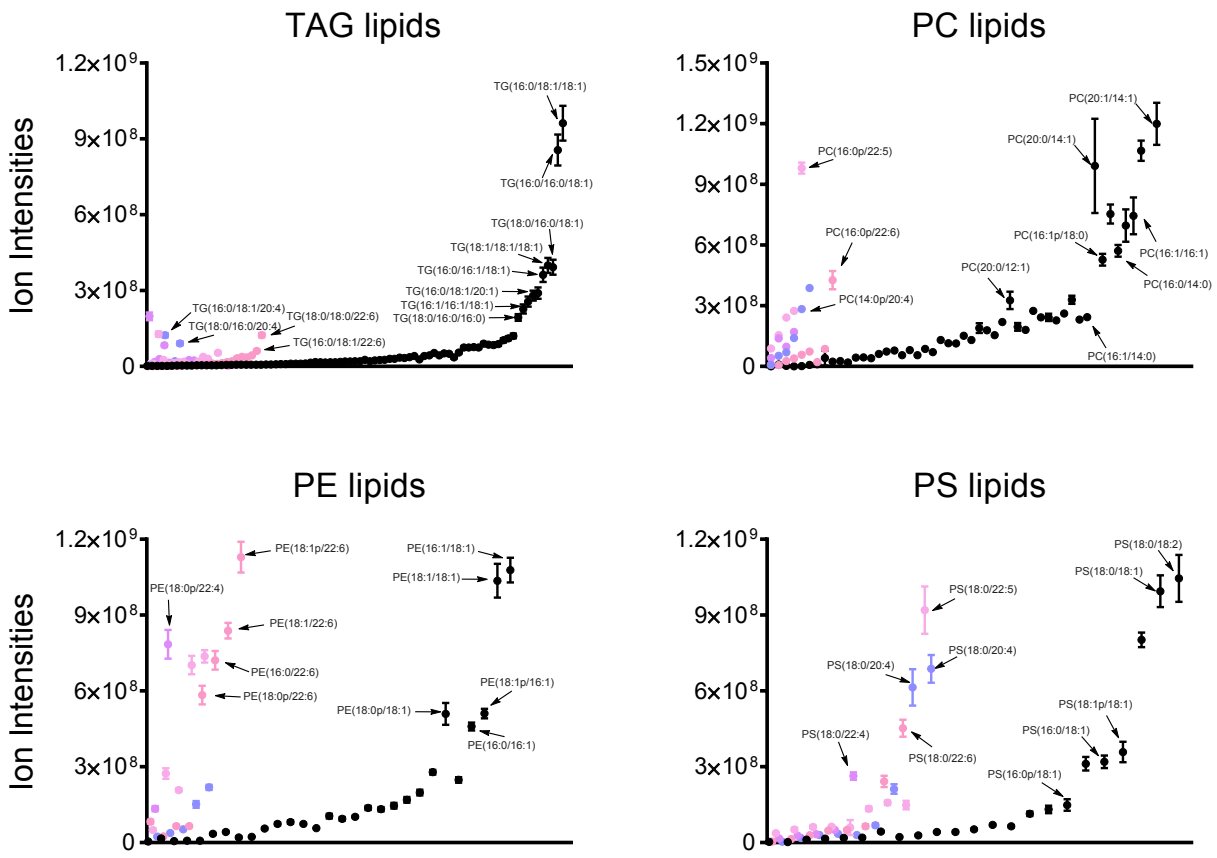

Figure S3. **Related to Figure 1. PUFA-TAGs are low abundance species compared with saturated counterparts in BMDMs**. Untargeted lipidomics profiling of DAGLβ WT BMDM lipid extracts was used to compare lipid species total ion intensities as a function of fatty acyl composition in each respective class. PUFA-TAGs were generally found at lower abundances compared with saturated TAG lipids. In contrast, the differences in abundance of PUFA and saturated phospholipid species appeared to be more evenly distributed (n=5 biological samples). All data shown are representative of two independent experiments (n=2 biologically independent experiments).

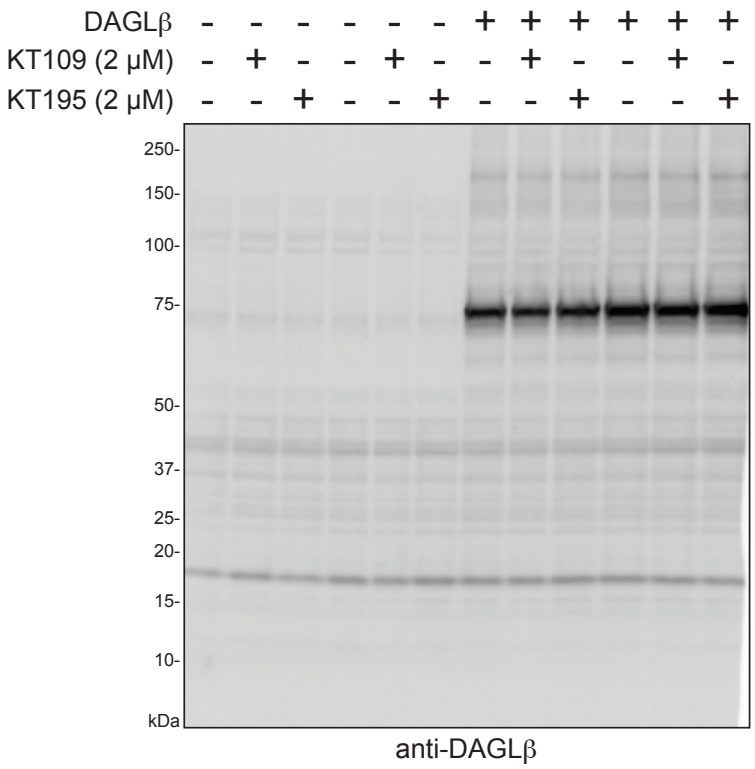

Figure S4. **Related to Figure 4. Western blot analysis of recombinant DAGLβ expression in HEK293T proteomes.** Western blot showed reproducible transient overexpression of recombinant mouse DAGLβ in HEK293T cells (anti-DAGLβ antibody). Treatment of recombinant DAGLβ-HEK293T membrane proteomes with compounds (2 μM of KT109 or KT195, 30 min, 37 °C) did not affect expression levels of recombinant DAGLβ protein compared with vehicle controls.