Supplementary Data

Diacylglycerol lipase-α and -β control neurite outgrowth in Neuro-2a cells through distinct molecular mechanisms. Jung, K.-M, Astarita, G., Thongkham, D. and Piomelli, D. Molecular Pharmacology.
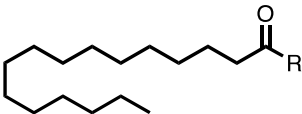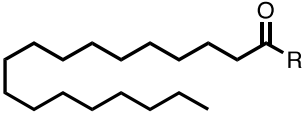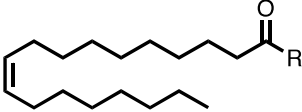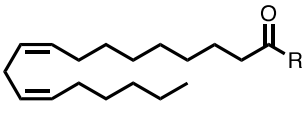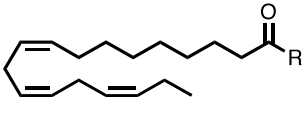
Supplementary Table 1

Supplementary Figure S1

Supplementary Figure S2

Supplementary Figure Legends

Supplementary Table I. MAG (R=glycerol) and FA (R=OH) levels (pmol/mg protein) in Neuro-2a cells following DGL-β overexpression (n=4).

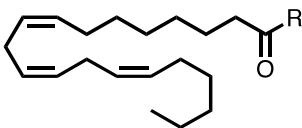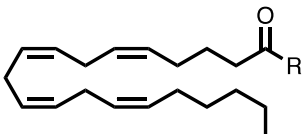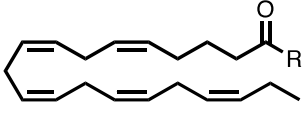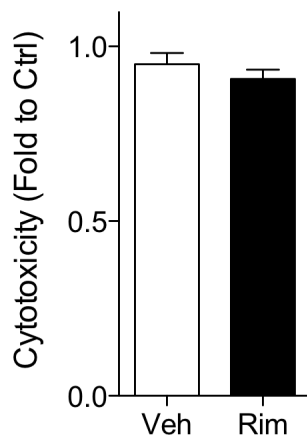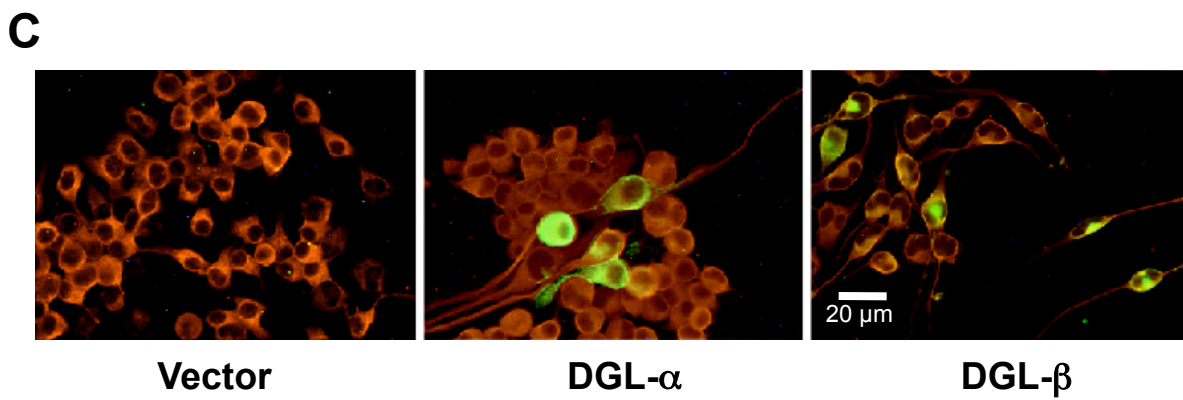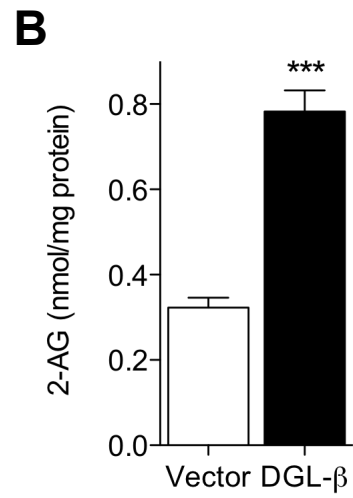| Acyl Chain	Structure	Lipid	m/z	Vector	DGL-β
16:0		MAG	353	278±8	278±10
		FA	255	293±7	309±7
18:0		MAG	381	51 ±9	41±2
		FA	283	414±19	448±27
18:1 Δ^9		MAG	379	206±18	259±11
		FA	281	158±1	181±5**
18:2 $\Delta^{9,12}$		MAG	377	29±3	50±4*
		FA	279	10±1	11±0.6
18:3 $\Delta^{9,12,15}$		MAG	375	9±2	21±7
		FA	277	0.8±0.1	0.5±0.1
20:3 $\Delta^{8,11,14}$		MAG	403	59±7	95±4**
		FA	305	3.5±0.1	4.0 ±0.1*
20:4 $\Delta^{5,8,11,14}$		MAG	401	322±23	782±49***
		FA	303	11±0.5	15±0.6**
20:5 $\Delta^{5,8,11,14,17}$		MAG	399	12±1	22±5
		FA	301	3.7±0.4	4.8±0.6
22:6 $\Delta^{4,7,10,13,16,19}$		MAG	425	9.6 ±0.5	20±1***
		FA	327	0.5±0.0	0.4±0.1

Values are means ± SEM, n=3-4. ***$P<0.001$, **$P<0.01$, *$P<0.05$ by two-tailed Student's t-test.

Supplementary Fig. S1

A

V   β

Anti-V5

— 100

— 75

— 50

Anti-DGLβ

— 75

Anti-Actin

— 50

B

C

Vector   DGL-α   DGL-β

20 µm
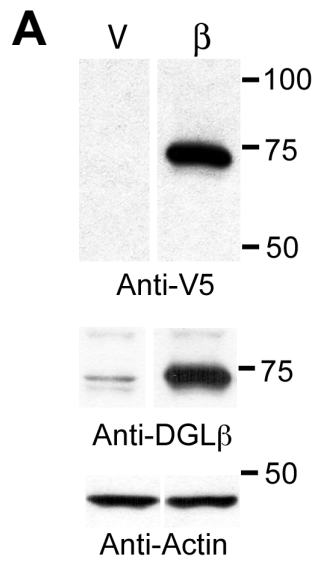
Supplementary Fig. S2

Legends for Supplementary Figure

Supplementary Figure S1. Exposure to rimonabant does not cause cytotoxicity in Neuro-2a cells. Neuro-2a cells were pre-treated with either vehicle (DMSO, Veh) or rimonabant (1 μM, Rim) for 10 min and then added with 20 μM RA for 24 hours at 37°C. Levels of lactate dehydrogenase (LDH) released into the cultured media were measured using cytotoxicity detection kit, LDH (Roche, Indianapolis, IN) and the values were normalized by no treatment control (n=8).

Supplementary Figure S2. Overexpression of DGL isoforms induces morphological changes of Neuro-2a cells. (A) Neuro-2a cells were transfected with control pEF6 vector (V) or DGL-β-V5-pEF6 (β). Cells were harvested 72 hours after transfection and the expression of DGL-β-V5 protein was assessed by western blot analyses using anti-V5 (top) or anti-DGL-β (middle) antibody (Jung *et al.*, 2007). Both antibodies recognized a band of approximately 70 kDa, which was highly expressed in DGL-β transfected cells. Actin (bottom) serves as a loading control. (B) The levels of 2-AG were determined under the same condition by a LC-MS method (n=4). (C) Neuro-2a cells were transfected with control pEF6 (Vector), DGL-α-V5-pEF6 (DGL-α) or DGL-β-V5-pEF6 (DGL-β). After 72 hours of transfection, cells were fixed and double-immunostained with a rabbit polyclonal anti-V5 (for DGL staining, green) and a mouse monoclonal anti-β-Tubulin (for β-Tubulin staining, red). Representative fluorescence microscope images were shown. ***P<0.001 by two-tailed *t*-test.