YMTHE, Volume *31*

Supplemental Information

MicroRNA137-loaded lipid nanoparticles regulate synaptic proteins in the prefrontal cortex

Michelle C. Palumbo, Milan Gautam, Alex Sonneborn, Kilsun Kim, Phillip A. Wilmarth, Ashok P. Reddy, Xiao Shi, Daniel L. Marks, Gaurav Sahay, Atheir I. Abbas, and Aaron Janowsky

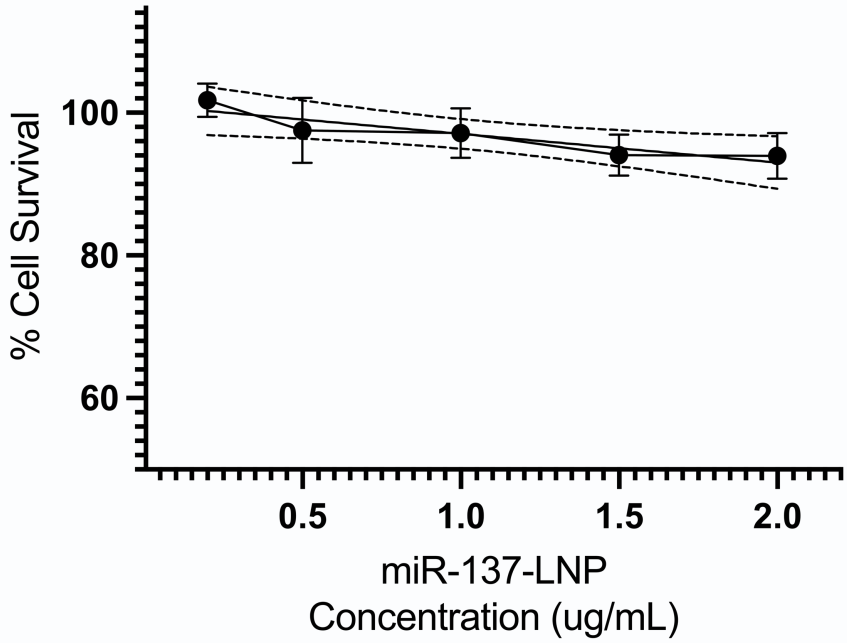

Figure S1. Lipid nanoparticle toxicity *in vitro*
MTT cell survival assay indicating minor toxicity after treatment with 0.5ug, 1ug, 1.5ug, or 2ug of miR137-LNPs in Neuro2A cell culture. n = 6, mean ± SEM

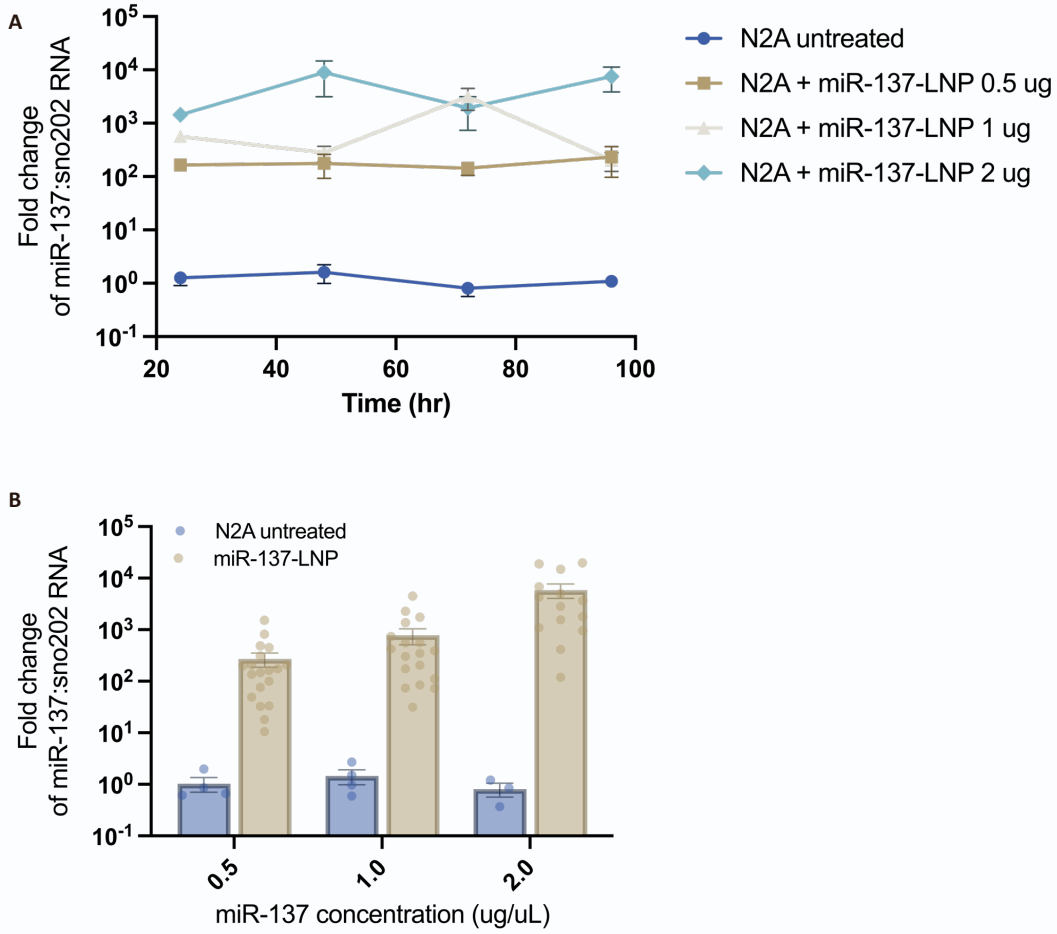

Figure S2. Lipid nanoparticle concentration and time course in neuronal cell culture
A) Time course of RT-qPCR fold change miR-137 expression to sno202RNA after treatment with **B)** 0.5ug, 1ug, or 2ug of miR-137-LNPs in Neuro2A cell culture. n = 3-19, mean ± SEM

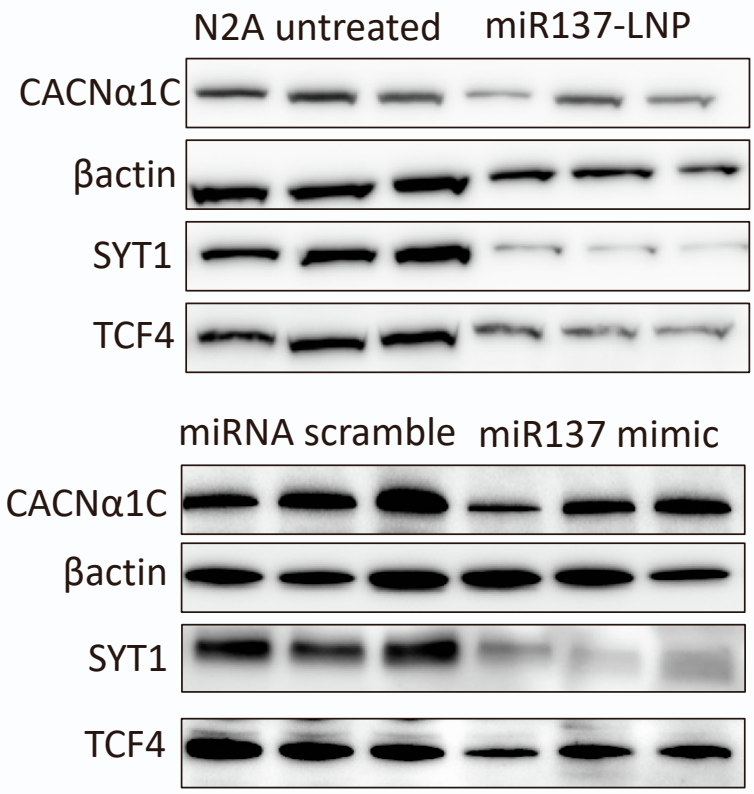

Figure S3. Immunoblots of synaptic proteins by treatment
Representative western blot images showing relative protein levels of CACNα1C, βactin, SYT1, and TCF4 in untreated N2A cells or cells treated with miRNA scramble, miR137 mimic, or miR137-LNP. Data are quantified in Figure 2D.

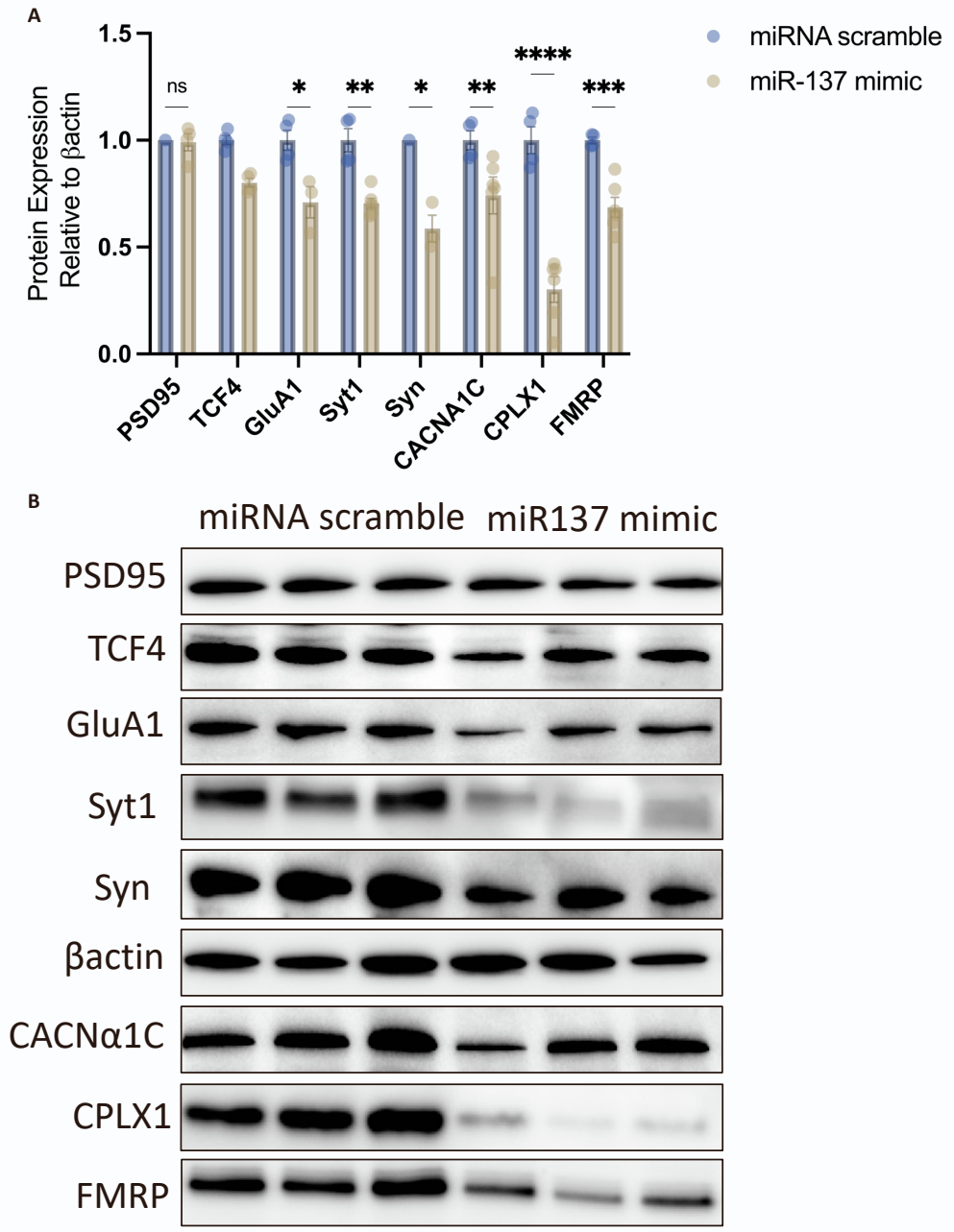

Figure S4. miR137 synaptic protein inhibition

A) Quantification and **B)** representative western blot images of treatment with miR137 mimic and miRNA scramble in N2A cells and relative expression of target synaptic proteins post synaptic density 95 (PSD95), transcription factor 4 (TCF4), glutamate receptor 1 (GluA1), synaptotagmin 1 (SYT1), synaptophysin (SYN), voltage gated calcium channel α1C (CACNα1C), complexin 1 (CPLX1), and fragile X mental retardation protein (FMRP). All protein levels are normalized to βactin in miRNA scramble. n = 3-6, mean ± SEM; ns, not significant: $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ and ****$p < 0.0001$.

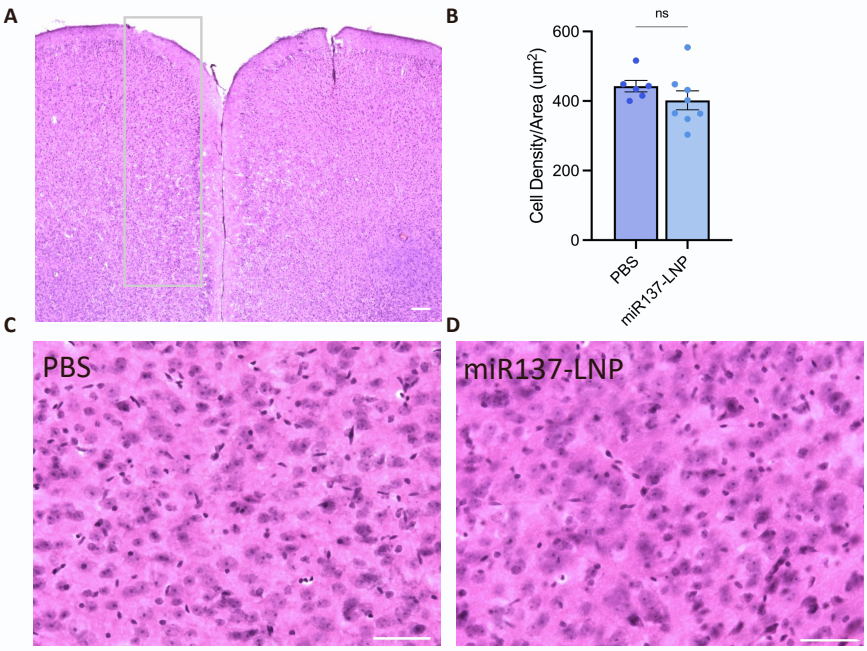

Figure S5. Cellular lipid nanoparticle toxicity *in vivo*
H&E stain from **A)** mouse prefrontal cortex brain tissue. scale bar: 200 µm **B**) Quantification of neuronal cell density per section area (μm^2) after 5 consecutive days of **C**) PBS or **D**) miR137-LNP treatments. scale bars: 100 µm. n = 2/treatment; mean ± SEM; ns, not significant

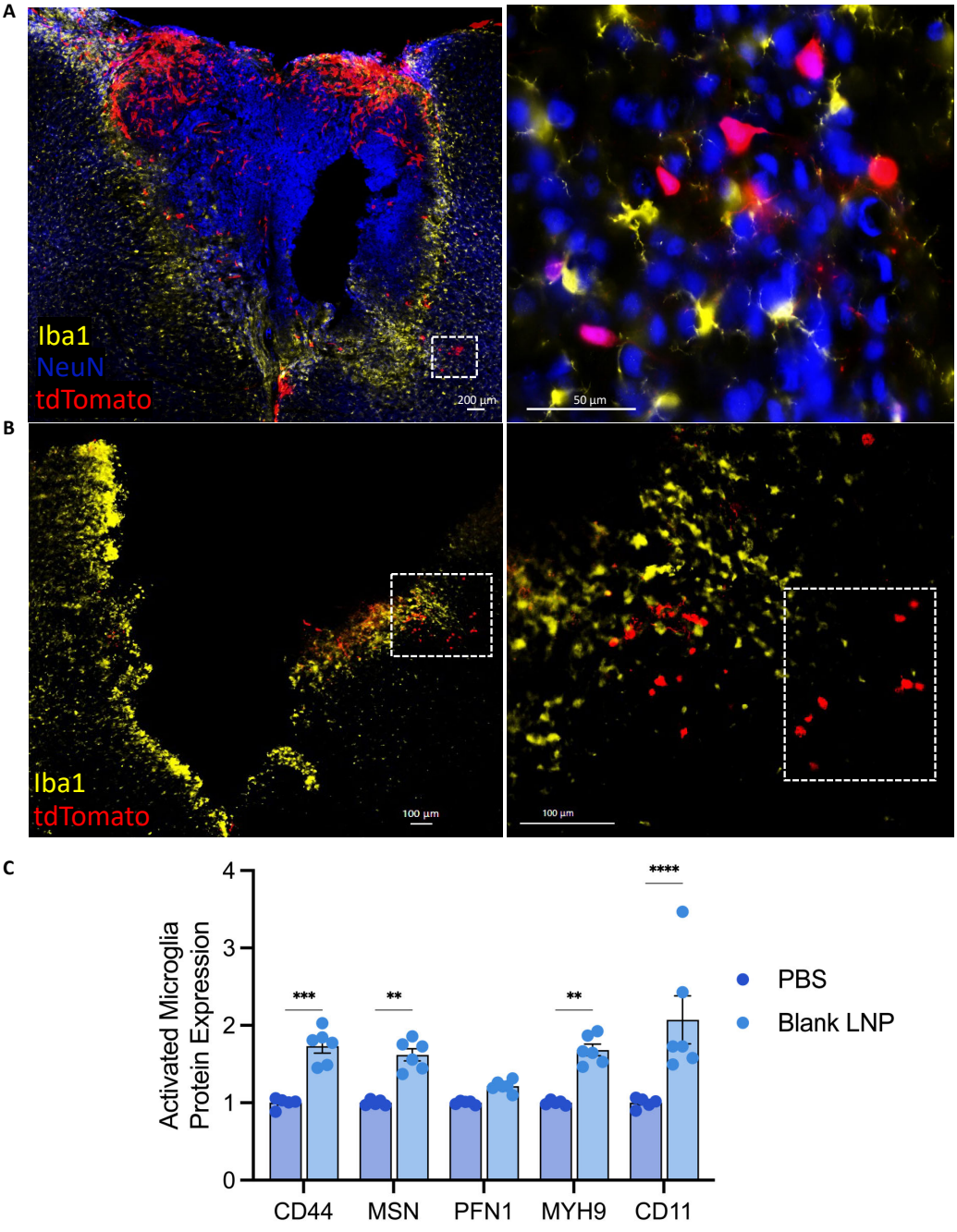

Figure S6. Microglia Quantification and Activation
Representative florescent images **A)** and **B)** of injection tract marks in the prefrontal cortex and adjacent quantified region (white box) for tdTomato (red), Iba1 (yellow), or NeuN (blue) co-labeling. **C)** Proteomic quantification of activated microglia protein expression comparing prefrontal cortex biopsies of animals who received PBS or blank LNPs: cluster of differentiation 44 (CD44), moesin (MSN), profilin 1 (PFN1), myosin heavy chain 9 (MYH9), and cluster of differentiation 11 (CD11) normalized to PBS. n = 5-6/group. mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$.

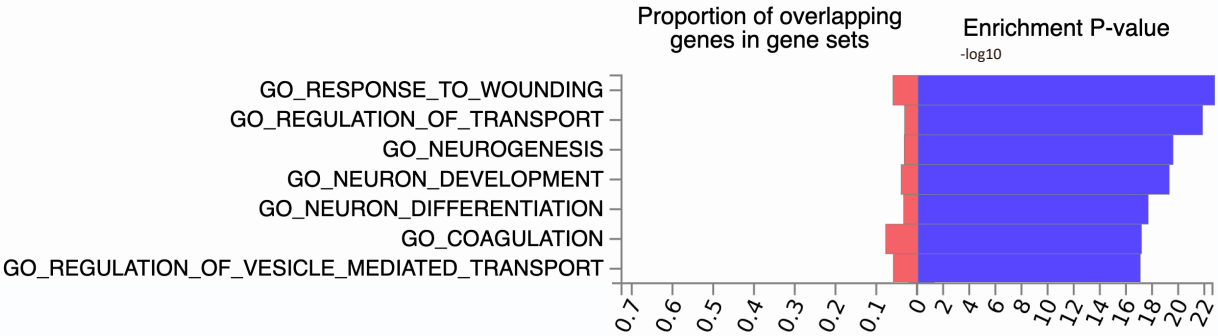

Figure S7. miR137-LNP GO Biological Process

Quantitative proteomics comparing prefrontal cortex biopsies of animals who received miR137-LNP or blank LNPs. GO biological process of enriched proteins from miR-137-LNPs involved in neuronal development. n = 5-6/group.

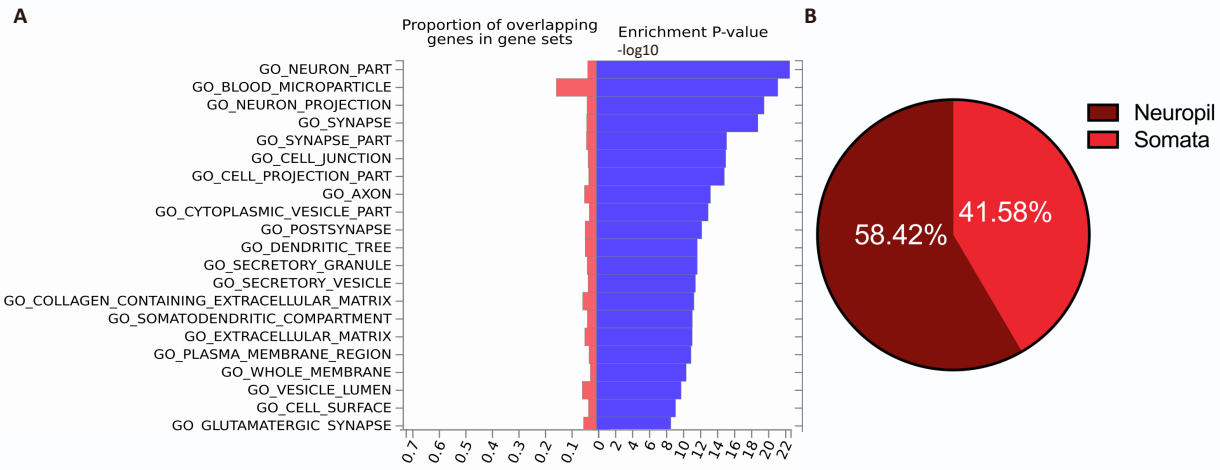

Figure S8. miR137-LNP GO Cellular Compartment

Quantitative proteomics comparing prefrontal cortex biopsies of animals who received miR137-LNP or blank LNPs. **A)** GO cellular compartment of enriched proteins from miR-137-LNPs located in neuronal synapses. **B)** The majority of DEP miR137-LNP proteins are translated in the neuropil compared to the somata. n = 5-6/group.

Table S1. Blank LNP Reactome pathway enrichment in the brain
Differentially expressed proteins (DEP) comparing blank LNPs to PBS treatments. False discovery rate (FDR).

LNP Reactome Pathways

GO-term	Term description	% network activation	% total DEP	FDR
MMU-168256	Immune system	17.95	19.14	2.92E-43
MMU-76002	Platelet activation, signaling and aggregation	29.64	4.93	6.18E-20
MMU-2262752	Cellular responses to stress	17.97	4.67	8.19E-10
MMU-166658	Complement cascade	44.0	1.45	1.91E-08
MMU-1280215	Cytokine signaling in immune system	16.54	4.14	1.12E-07

Table S2. Blank LNP biological process enrichment in the brain
Differentially expressed proteins (DEP) comparing blank LNPs to PBS treatments. False discovery rate (FDR).

LNP Biological Process

GO-term	Term description	% network activation	% total DEP	FDR
GO:0033993	Response to lipid	12.04	6.91	4.92E-06
GO:0006629	Lipid metabolic process	11.14	7.56	3.61E-05
GO:0044255	Cellular lipid metabolic process	11.17	5.86	0.00042
GO:0006869	Lipid transport	13.57	2.30	0.0049
GO:0006644	Phospholipid metabolic process	12.71	2.50	0.0081