

Manuscript Title: *Toxoplasma gondii* infection induces extracellular vesicle miRNAs in synaptic plasticity and neural mechanisms

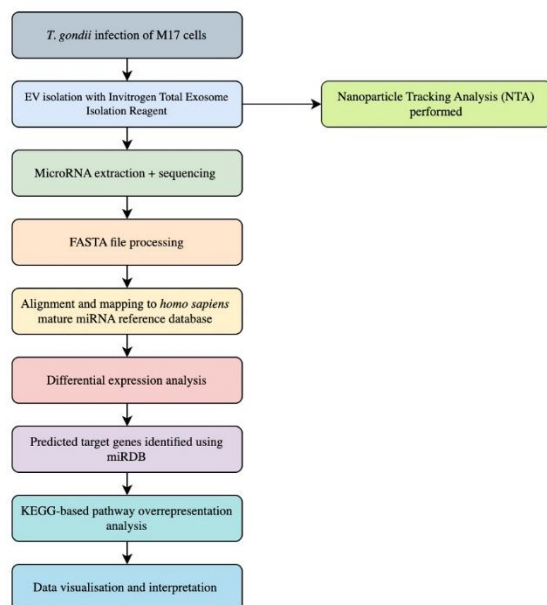
Manuscript Author:

Poppy Cairney¹, Elton Rosas de Vasconcelos², and Glenn A. McConkey¹

¹School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom.

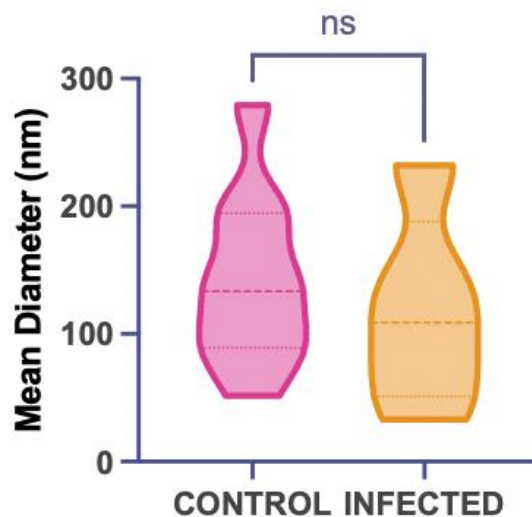
²Faculty of Biological Sciences, LeedsOmics, University of Leeds, Leeds LS2 9JT, United Kingdom.

Correspondence to: Dr. Glenn McConkey, School of Biology, University of Leeds, Leeds LS2 9JT, United Kingdom. E-mail: G.A.McConkey@Leeds.ac.uk



Supplementary Figure 1. Workflow for miRNA-Seq Data Processing and Analysis. This flowchart outlines the stepwise pipeline used for miRNA sequencing analysis. BE(2)-M17 cells were cultured, with half the samples infected with *Toxoplasma gondii* (1:1 MOI). Nanoparticle Tracking Analysis (NTA) was performed to confirm EV size and concentration. Small RNA was extracted (n=6), followed by library preparation and sequencing. The raw sequencing data underwent preprocessing, quality control, and alignment to the *Homo sapiens* miRNA reference database (mature.fa). Read counts were obtained using featureCounts, and differential expression analysis was performed using DEseq2 package in

R. The p-value cutoff applied was 0.1. Target prediction was conducted using miRDB, and KEGG-based pathway enrichment analysis was carried out using ShinyGo. Visualisation techniques, including heatmaps and scatterplots, were used to interpret differentially expressed miRNAs and their associated pathways.

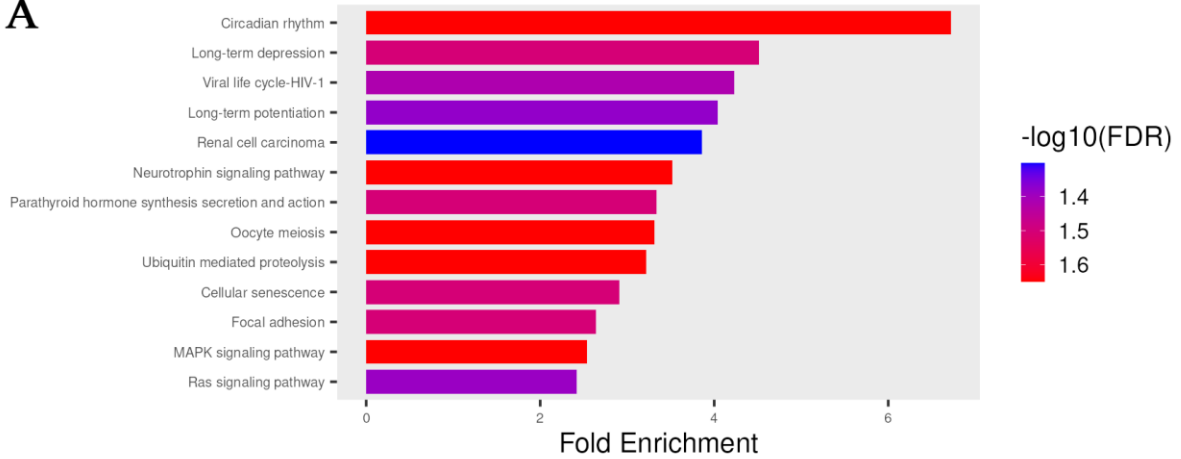


Supplementary Figure 2. *Exosomal vesicle size distribution in control and infected samples.*

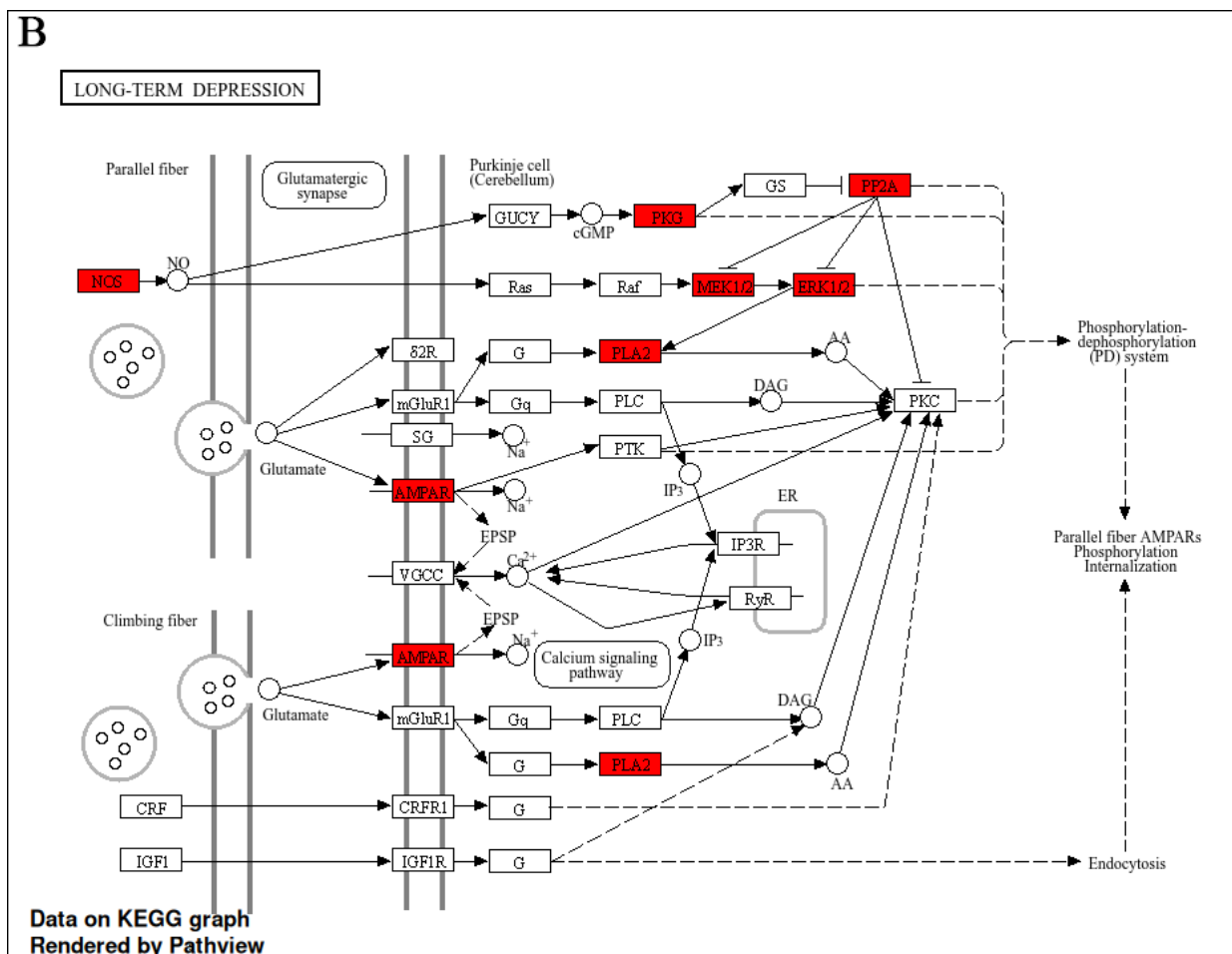
Violin plot showing the mean diameter (nm) of exosomes isolated from control and *Toxoplasma gondii*-infected M17 cultures, measured using nanoparticle tracking analysis (NTA). Statistical comparison was performed using an unpaired two-tailed t -test ($t = 0.6261$, $df = 13$), yielding a p -value of 0.5421, indicating no significant difference in vesicle size between conditions. Median and quartiles are indicated within each distribution.

Full size of **Figure 2**.

A

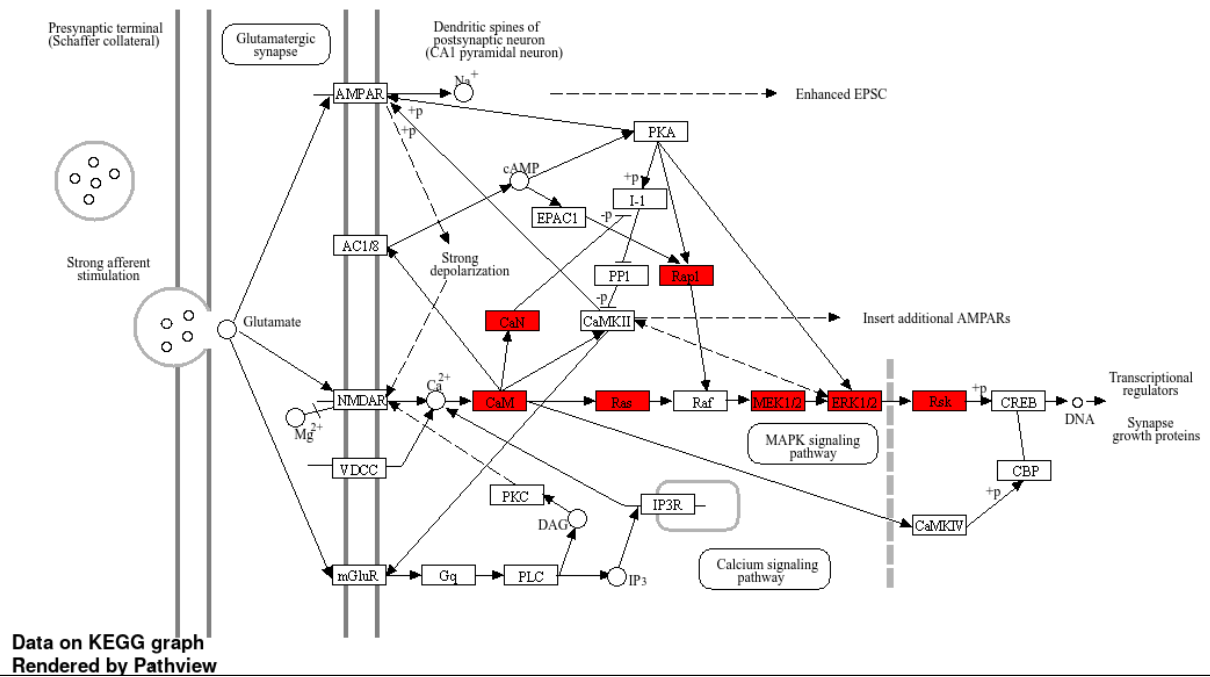


B



C

LONG-TERM POTENTIATION



D

NEUROTROPHIN SIGNALING PATHWAY

Neuron

MAPK signaling pathway

Cellular differentiation
Cell survival
Retrograde transport
Axonal outgrowth

Ubiquitin mediated proteolysis

CREB

DNA

Bcl-2

Retrograde transport

Cell survival

Plasticity

Long-term potentiation

Regulation of actin cytoskeleton

Membrane ruffling
Axonal outgrowth
Axonal guidance
Axon patterning
Synapse formation

Cell cycle arrest

Apoptosis

Cell survival

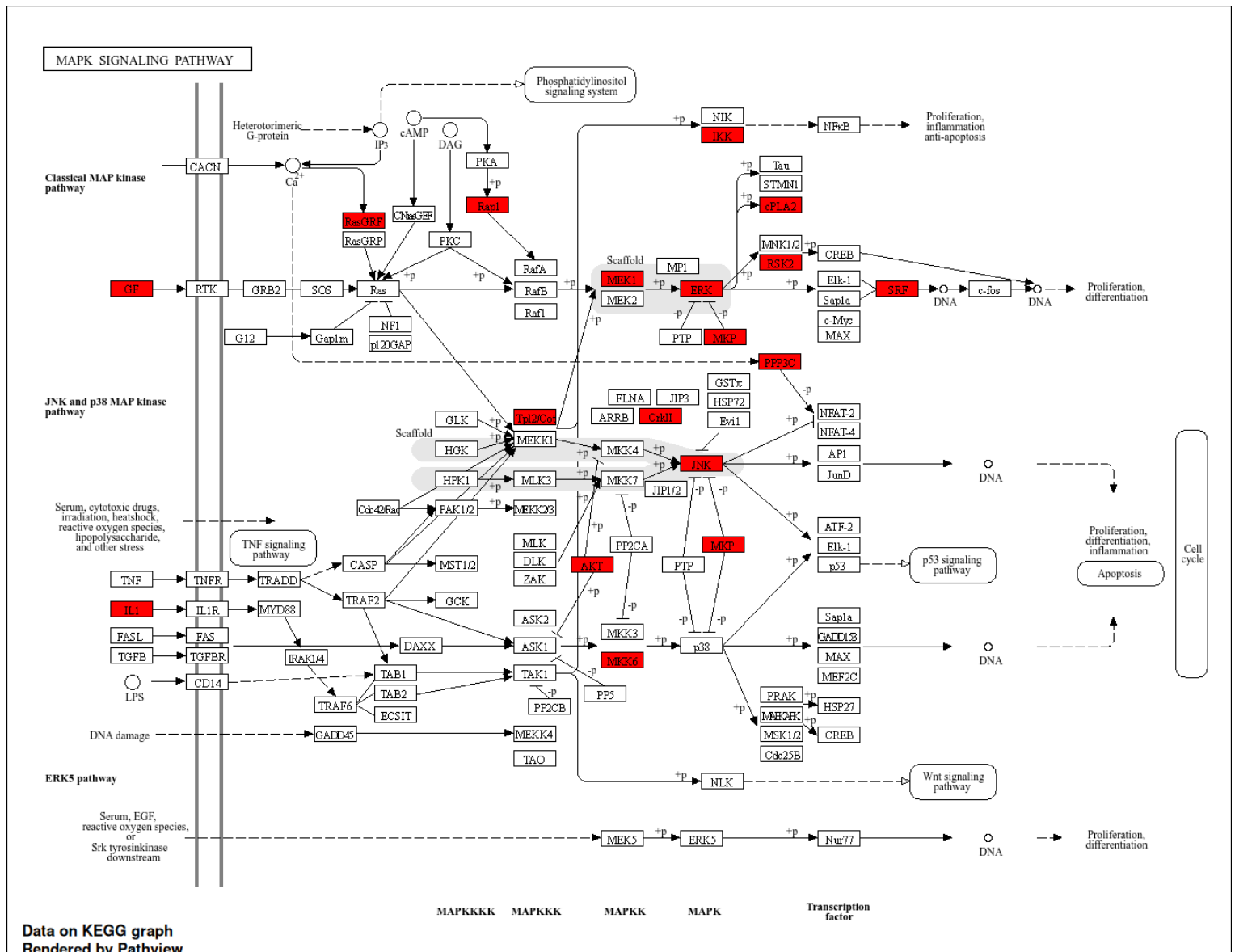
Extracellular domain

Sortilin

Data on KEGG graph
Rendered by Pathview

Data on KEGG graph
Rendered by Pathview

MAPK Signaling



Ras signaling

