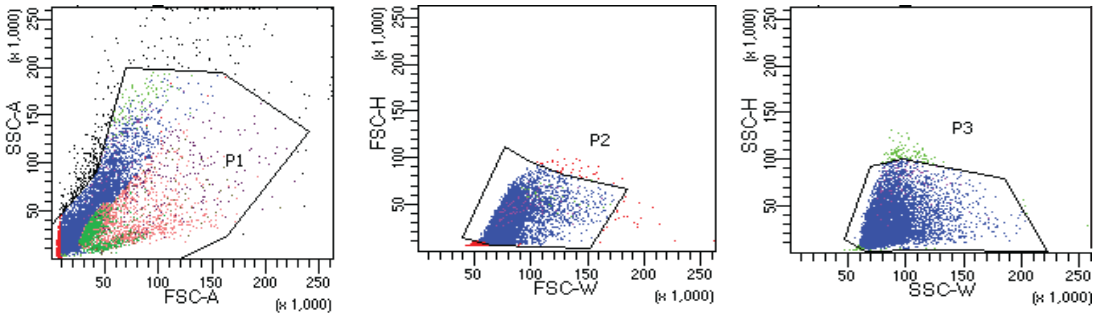
**iScience, Volume *25***

## Supplemental information

# Downregulation of neurodevelopmental gene expression in iPSC-derived cerebral organoids upon infection by human cytomegalovirus

**Benjamin S. O'Brien, Rebekah L. Mokry, Megan L. Schumacher, Kirthi Pulakanti, Sridhar Rao, Scott S. Terhune, and Allison D. Ebert**

A

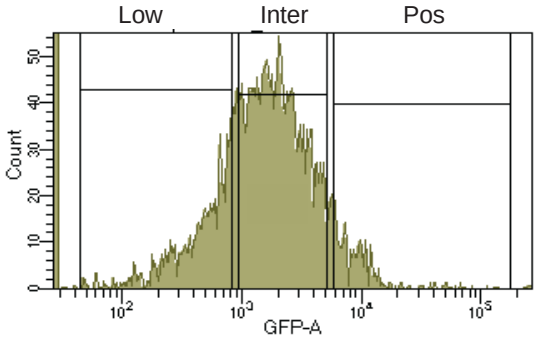



B

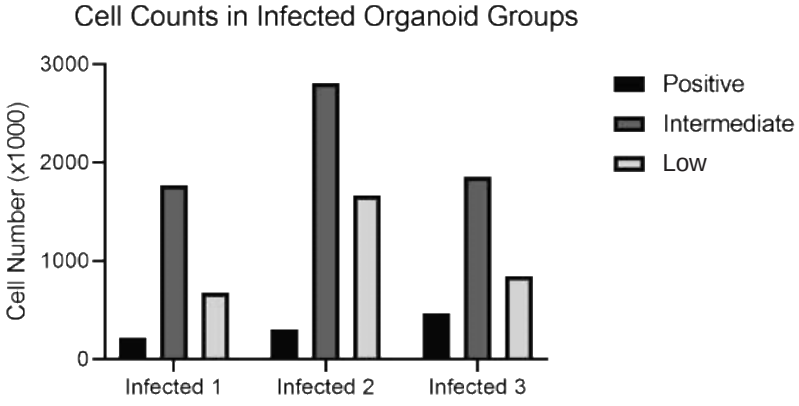
Tube: 141516

| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 10,000 | #### | 100.0 |
| P1 | 9,628 | 96.3 | 96.3 |
| P2 | 8,195 | 85.1 | 82.0 |
| P3 | 8,053 | 98.3 | 80.5 |
| P4 | 67 | 0.8 | 0.7 |
| P5 | 3,019 | 30.2 | 30.2 |
| gfp int | 1,774 | 58.8 | 17.7 |
| gfp pos | 222 | 7.4 | 2.2 |
| gfp low | 678 | 22.5 | 6.8 |

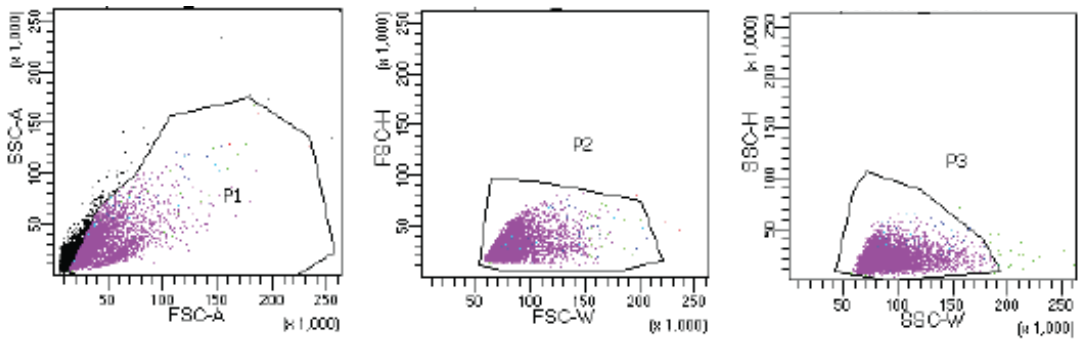
C



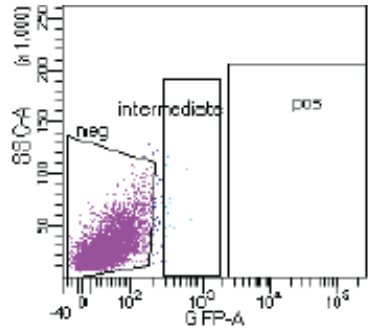

D



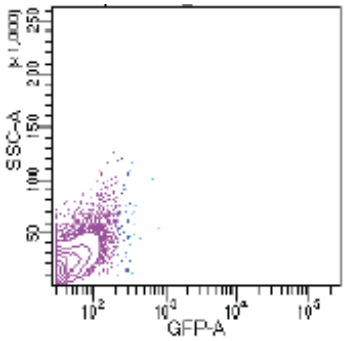
A


SSC-A
(x 1,000)
P1
FSC-A
(x 1,000)
FSC-H
P2
FSC-W
SSC-H
P3
SSC-W

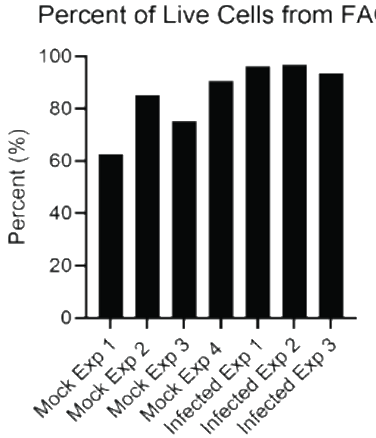
B


SSC-A
neg
intermediate
pos
GFP-A

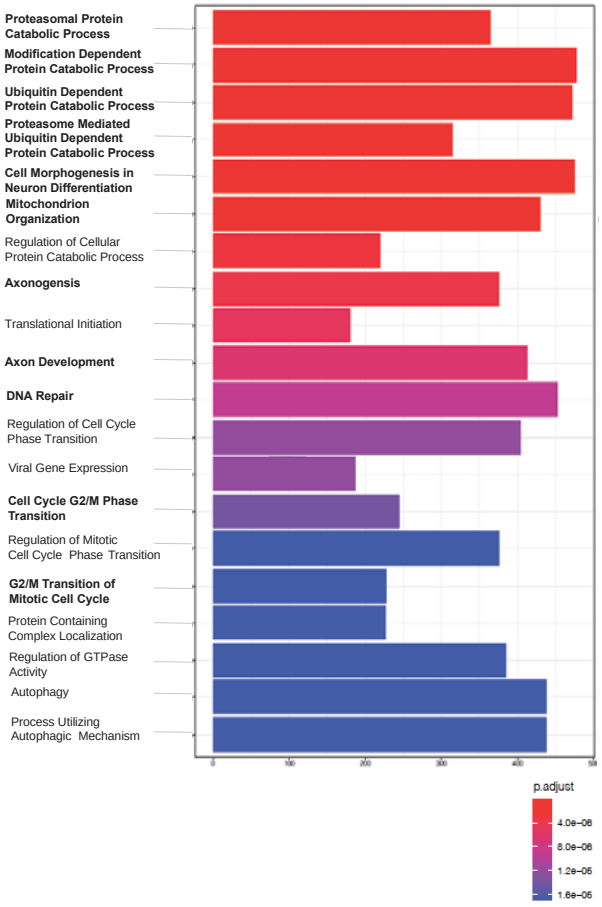
C


SSC-A
GFP-A

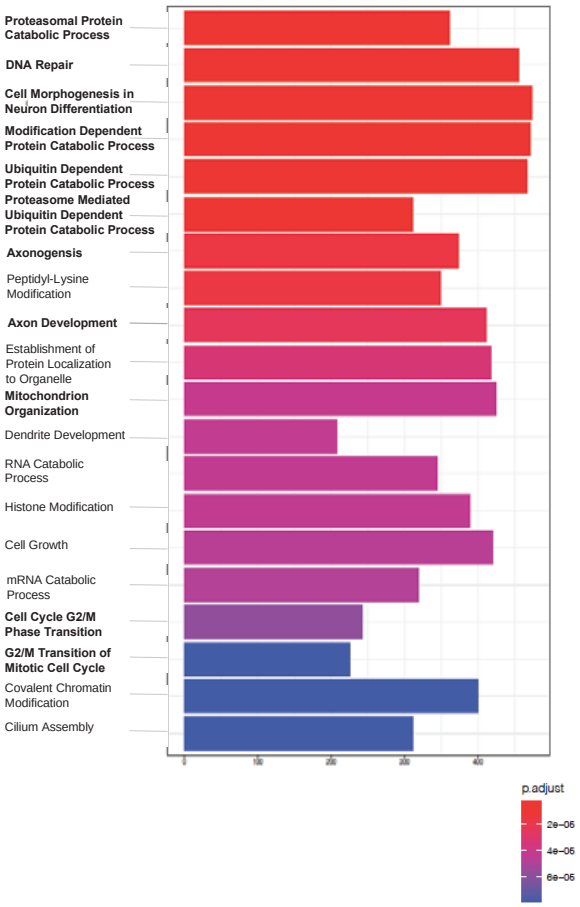
D


Percent of Live Cells from FACs
Percent (%)
Mock Exp 1
Mock Exp 2
Mock Exp 3
Mock Exp 4
Infected Exp 1
Infected Exp 2
Infected Exp 3

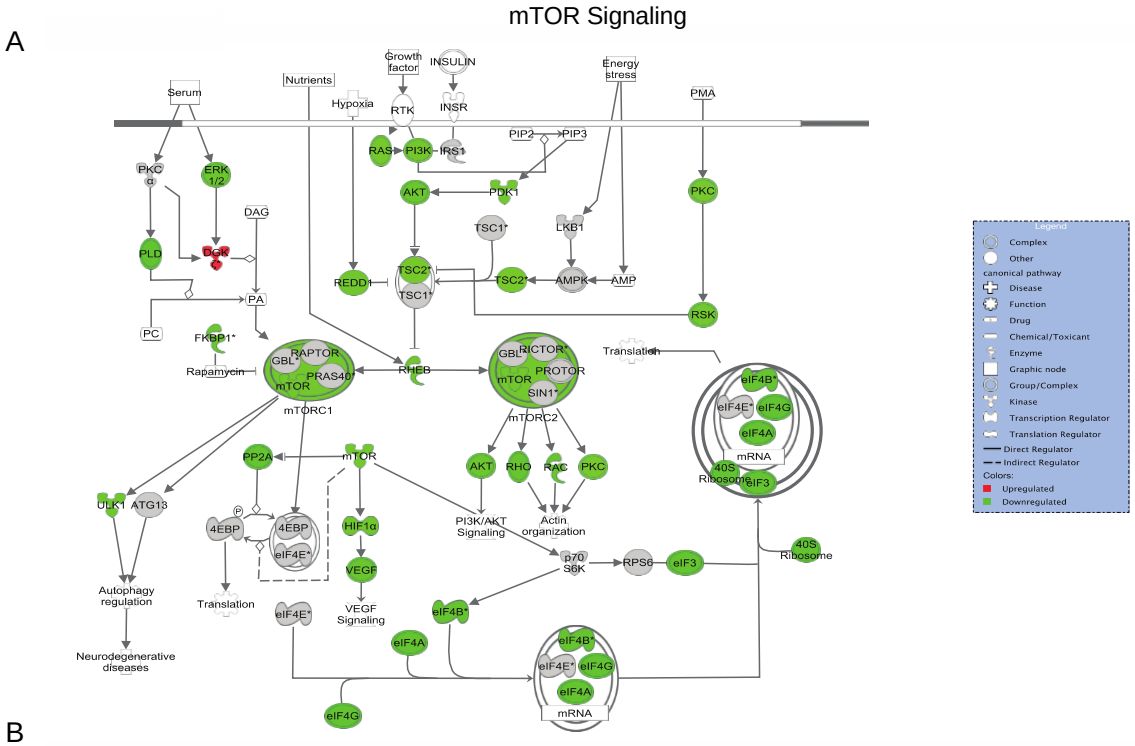
## GFP (+) vs Mock



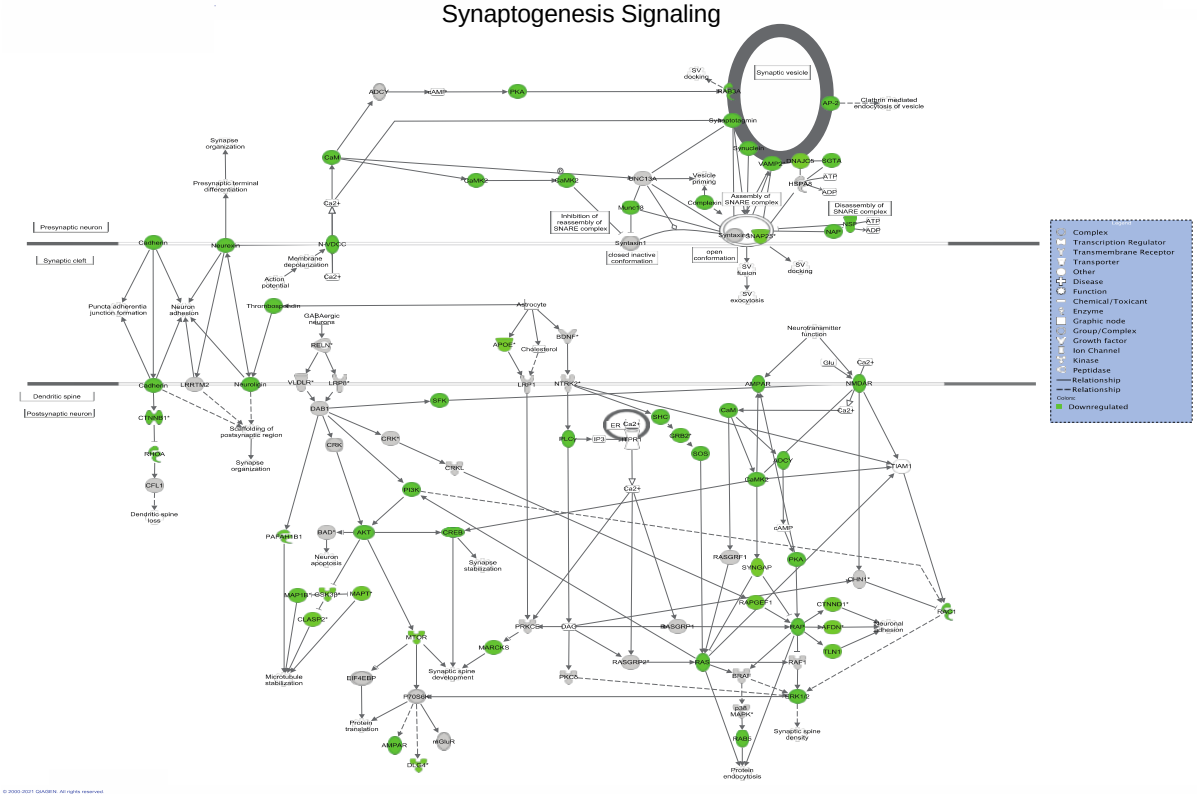

## GFP (Low) vs Mock



A

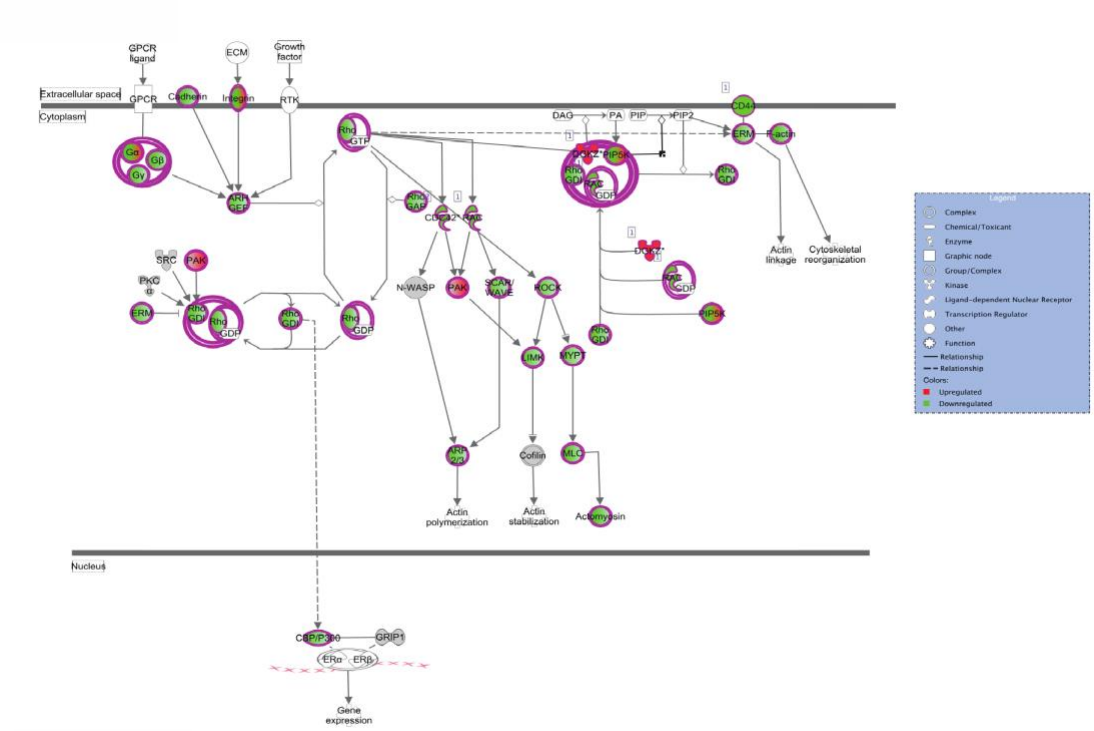

mTOR Signaling
Growth factor
INSULIN
Energy stress
Nutrients
Serum
Hypoxia
RTK
INSR
PMA
PIP2
PIP3
RAS
PI3K
IRS1
PKC
ERK 1/2
AKT
PDK1
DAG
TSC1*
LKB1
PLD
EGR
REDD1
TSC2*
TSC1*
AMPK
AMP
PA
RSK
PC
FKBP1*
RAPTOR
GBL
PRAS40
mTOR
RHEB
RICTOR*
PROTOR
SIN1*
Translation
Rapamycin
mTORC1
mTORC2
eIF4B*
eIF4E*
eIF4G
eIF4A
mRNA
40S Ribosome
eIF3
PP2A
mTOR
AKT
RHO
RAC
PKC
ULK1
ATG13
4EBP
HIF1α
VEGF
PI3K/AKT Signaling
Actin organization
p70 S6K
RPS6
Autophagy regulation
Translation
VEGF Signaling
Neurodegenerative diseases
Legend
Complex
Other
canonical pathway
Disease
Function
Drug
Chemical/Toxicant
Enzyme
Graphic node
Group/Complex
Kinase
Transcription Regulator
Translation Regulator
Direct Regulator
Indirect Regulator
Colors:
Upregulated
Downregulated

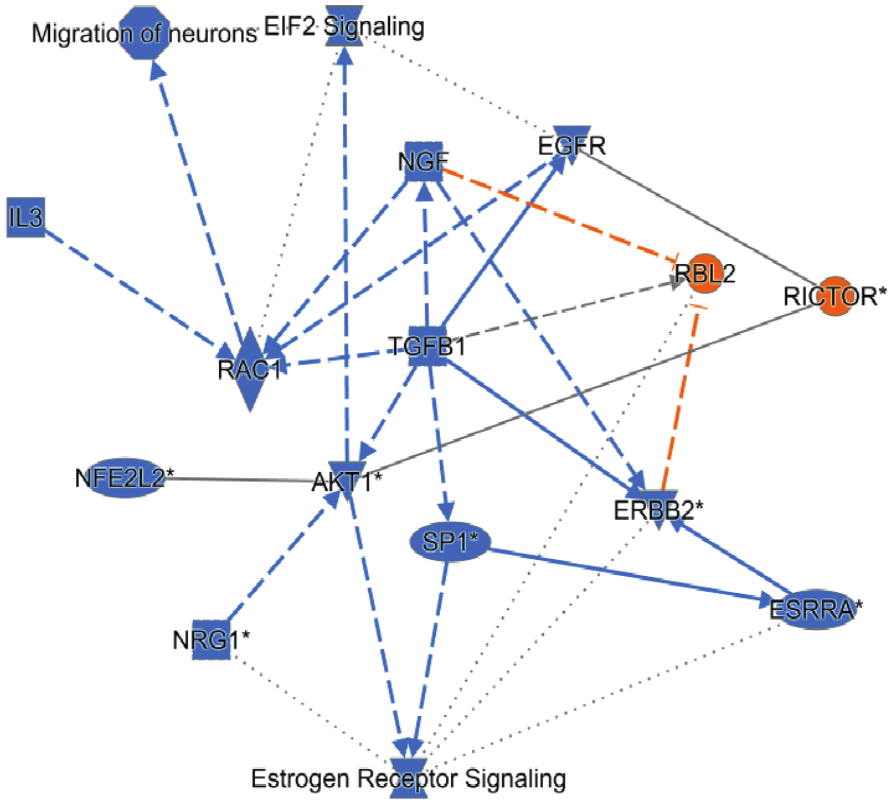
B


Synaptogenesis Signaling
Synaptic vesicle
Presynaptic neuron
Synaptic cleft
Dendritic spine
Postsynaptic neuron
Complex
Transcription Regulator
Transmembrane Receptor
Transporter
Other
Disease
Function
Chemical/Toxicant
Enzyme
Graphic node
Group/Complex
Growth factor
Ion Channel
Kinase
Peptidase
Relationship
Relationship
Colors:
Downregulated

C

RhoGDI Signaling






Predicted Inhibition
Predicted Activation
Leads to Inhibition
Leads to Activation
Effect not predicted
* Key Upstream Regulator

Dotted Line Indirect Effect
Solid Line Direct Effect

**Supplemental Figures and Table Legends**

**Figure S1. Representative FACs traces from an HCMV-TB40EGFP infected organoid, Related to Figure 1.** (A) Set of three scatter analysis plots used to determine the live cell population within the organoid; live cell gates were set based on the uninfected condition. (B) Table showing relative cell counts within each gated population. (C) Plot of GFP signal intensity against total number of sorted cells; GFP gates were set based on previous infected organoid sorting. (D) Relative number of cells within each infected subpopulation shown for each replicate.

**Figure S2. Representative FACs traces from an uninfected organoid, Related to Figure 1.** (A) Set of three scatter analysis plots used to determine the live cell population within the organoid. Live cell gates were set based on the uninfected condition. (B-C) Side scatter versus GFP plot in an uninfected organoid showing lack of GFP signal. (D) Percentage of live cells across all organoids sorted for sequencing.

**Figure S3. Additional gene ontology analysis for GFP (+) vs. Mock and GFP (Low) vs. Mock, Related to Figure 2 & 3.** Ontology conducted using the same 3,000 gene list and criteria as Figure 3A with the platform DAVID instead of G-profiler. Results shown are the top 20 most significant terms.

**Figure S4. Network diagrams generated from IPA on GFP (+) vs. Mock summarize three key affected canonical pathways that were differentially affected by infection, Related to**

**Figures 3 & 4.** (A) mTOR signaling -log(adj p-value) = 17.479 with the majority of pathway components downregulated. (B) Synaptogenesis signaling pathway -log(adj p-value) = 16.367. The majority of pathway components were downregulated. (C) RHOGDI signaling -log(adj p-value) = 11.123. There was a mixture of upregulated and downregulated pathway components.

**Figure S5. Ingenuity pathway core analysis diagram for GFP (+) vs. Mock, Related to Figure 4.** Node diagram generated using IPA to connect major affected pathways and functional outcomes within the GFP (+) samples to regulators using the same gene list from Figure 4A. Solid lines indicate direct interaction, and dashed lines indicate indirect interaction.

| Gene Identifier | Forward 5'-3' | Reverse 3'-5' |
|---|---|---|
| GAPDH | GTGGACCTGACCTGCCGTCT | GGAGGAGTGGGTGTCGCTGT |
| CAMKV | AAGATGAGAGCAGGACACCC | CAAGCAGGCTTGCAGTCAGA |
| KCNF1 | GGGTTGGGTGTGGAGTTTTG | GGATTTAACCCAATCACCATGAC |
| CACNA1G | GCTGGGTCGACATCATGTACTTTG | CTGAGAACTGCGTGGCAAACC |
| FEZF2 | GTGGTGGAATTCGCCGCCGCCATGGCCAGC TCAGCTTCCCTGGAGACCATGGTG | TGCTGGATATCAGCTCTGAACTGTCCT GGCTAGGTCCTTTGCTGA |
| EMX1 | AGCCCCGTCTTAATGCAACA | CTAGGATTGCGGGGCTAGTG |
| CACNA1C | GCAACGGCTGGAACCTACTA | GCAACGGCTGGAACCTACTA |
| FOXG1 | CGTTCAGCTACAACGCGCTCA | CAGATTGTGGCGGATGGAGTT |
| GJA1 | TACCAAACAGCAGCGGAGTT | TGGGCACCACTCTTTTGCTT |
| DMRTA2 | GCCTGCCTACGAAGTCTTTGGCTCGGTTT | CGTCTTGGGAAACAGATCAAACTTCTG |
| UL44 | GCCCGATTTCAATATGGAGGTCAG | CGGCCGAATTCTCGCTTTC |

| | | |
|---|---|---|
| UL99 | GTGTCCCATTCCCGACTCG | TTCACAACGTCCACCCACC |
| UL122 | ACCTGCCCTTCACGATTCC | ATGGTTTTGCAGGCTTTGATG |
| UL123 | GCCTTCCTAAGACCACCAAT | ATTTTCTGGGCATAAGCCATAATC |

**Table S7 qPCR Primer Set Table, Related to STAR Methods.** Primer sets used for qPCR in Figure 5 and 7.