Supplementary Material

Competing endogenous RNAs in human astrocyte: crosstalk and interacting networks in response to lipotoxicity

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

A

B

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MEG3     55201 CCCTCTCTTTGTCCCTCCCCAGTTCCTGACCTGGCCATCCCGGGGTGCCCTTGACCAGCCCCGTGTCTCCTCAGGGTGTCCCAGCACCAGCCTGGCACAGAGTGGGGCTCAGTTAGAGTA 55320
MEG3-202 55201 CCCTCTCTTTGTCCCTCCCCAGTTCCTGACCTGGCCATCCCGGGGTGCCCTTGACCAGCCCCGTGTCTCCTCAGGGTGTCCCAGCACCAGCCTGGCACAGAGTGGGGCTCAGTTAGAGTA 55320
MEG3-236 55201 --------------------------------------------------------------------------GGTGTCCCAGCACCAGCCTGGCACAGAGTGGG-------------- 55320
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C

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>MEG3-202_ENST00000398461_Exon1
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```
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TTTGAAATGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAGTGGCGTGATCTCGGCTCACTGCAACCTCTGCCTTCCGGGTTCAAGCAATTCTCCCACCTC
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ATAGAGCACGCCTGATGTTTCTAAATGAGAAGACTTTCAATAGTAATGAAGAATCCATGGCACTCTCCTCACCCTCAAACACATGGCAGTCATTCACATACA
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TTTTGTGCCCAAGGCTCCTGGAGCCCTAAAAACTTTCAAAAGTTAACTCCCCACGTCCCCATCCTGCTTGGGTTTCTGGACTTTTCTGAGGCACCGGCAGAG
GGGTCTCGTTGCTCCCTTGAGTGTAGGGGCAGCCCTTTAACCTGGCTCCTTGAGTCCCTGCTTTTTCTGCTTCTGTTGCCTTCTTCCTCGTCTTCCTCTCTC
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CGAAGAGTTCTAGGTGCAAAGGCTGGAGGGTGGAATTCATTTTTGAGAGGTGTGTGAGCAGCTTCCGACCCCTGCCCCATTTGAACGGGGGCCTTGCTGGTC
GCGTCCCTGCATTCACCCGCGCGGCCATCCCGTCATCCAACAGTTGATCCTAACTGAGCACGCCCACGGCCCTGGTCTGGCCTGGGCACCGGCCACCGTAGC
CCATCCCTTGATGGCCTCTGTGTCCCCAGGAGGGCGGGCCGGGGGGTTGCCCAGGGGCTGGAGCAGTGGACTGTGGCTCCATAGAGGTAGGCCGGAGGGTGT
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GAGGGCAGATTCAAGCTATCCCCAGGGCTCTGCTCTGGTCGGAGCCAGCCCCTTCTCCCTCTCTGCCTTCCCCGCCCCATTCCTGATGCTGAACTGTTCTGG
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TGCCTGTTTCCAGCAGGTTCCCAGTGCCCCCGACAAGCCCCTGCTGGTGTCTCCATCTCCTGCCAAGCATCCTCCAGTGCCTCCTCCTGTGGGCCTGGCCTC
AGGGCTATGGACAGACTCCTGTCCCATCCCAGAGACCCCTCGTGATCGTGCCCTG
```

`>MEG3-202_ENST00000398461_Exon2`

```
GCACGTGGGCCGTGGCCCGGCTGGGTCGGCTGAAGAACTGCGGATGGAAGCTGCGGAAGAGGCCCTGATGGGGCCCACCATCCCGGACCCAAGTCTTCTTCC
TGGCGGGCCTCTCGTCTCCTTCCTGGTTTGGG
```

`>MEG3-202_ENST00000398461_Exon3`

```
CGGAAGCCATCACCTGGATGCCTACGTGGGAAGGGACCTCGAATGTGGGACCCCAGCCCCTCTCCAGCTCGAAATC
```

`>MEG3-202_ENST00000398461_Exon4`

```
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ACTGATGGACGCCGCTGACCTGGGGTCAGACCCGTGGGCTGGACCCCTGCCCACCCCGCAGGAACCCTGAGGCCTAGGGGAGCTGTTGAGCCTTCAGTGTCT
GCATGTGGGAAGTGGGCTCCTTCACCTACCTCACAGGGCTGTTGTGAGGGGCGCTGTGATGCGGTTCCAAAGCACAGGGCTTGGCGCACCCCACTGTGCTCT
CAATAAATGTGTTTCCTGTCTTAAC
```

D

`>MEG3-236_ENST00000648820_Exon1`

```
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```

`>MEG3-236_ENST00000648820_Exon2`

```
GGTCTCTCCTCAGGGATGACATCATCCGTCCACCTCCTTGTCTTCAAGGACCACCTCCTCTCCATGCTGAGCTGCTGCCAAGGGGCCTGCTGCCCATCTACA
```

```
CCTCACG
```

```
>MEG3-236_ENST00000648820_Exon3
```

```
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ACCAGCCAGCTGTCCCTCTTACCTAAAGACTTAAACCAATGCCCTAGTGAGGGGGCATTGGGCATTAAGCCCTGACCTTTGCTATGCTCATACTTTGACTCT
ATGAGTACTTTCCTATAAGTCTTTGCTTGTGTTCACCTGCTAGCAAACTGGAGTGTTTCCCTCCCCAAGGGGGTGTCAGTCTTTGTCGACTGACTCTGTCAT
CACCCTTATGATGTCCTGAATGGAAGGATCCCTTTGGGAAATTCTCAGGAGGGGGACCTGGGCCAAGGGCTTGGCCAGCATCCTGCTGGCAACTCCAAGGCC
CTGGGTGGGCTTCTGGAATGAGCATGCTACTGAATCACCAAAGGCACGCCCGACCTCTCTGAAGATCTTCCTATCCTTTTCTGGGGGAATGGGGTCGATGAG
AGCAACCTCCTAGGGTTGTTGTGAGAATTAAATGAGATAAAAGAGGCCTCAGGCAGGATCTGGCATAGAGGAGGTGATCAGCAAATGTTTGTTGAAAAGGTT
TGACAGGTCAGTCCCTTCCCACCCCTCTTGCTTGTCTTACTTGTCTTATTTATTCTCCAACAGCACTCCAGGCAGCCCTTGTCCACGGGCTCTCCTTGCATC
AG
```

```
>MEG3-236_ENST00000648820_Exon4
```

```
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ACGCTCACAAAG
```

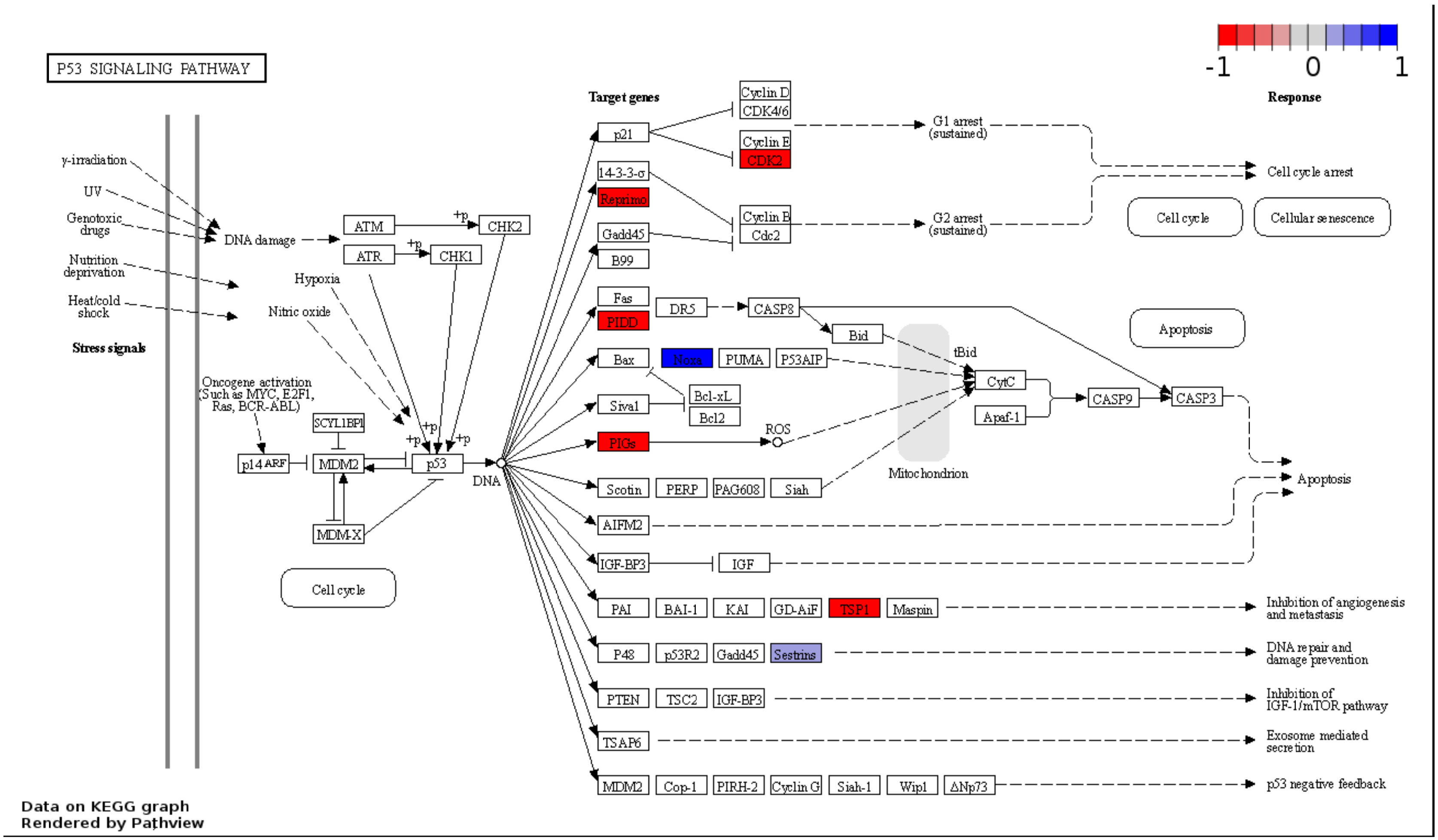
```
>MEG3-236_ENST00000648820_Exon5
```

```
GGCTAATCTCGGGCCTTGTCGAAGGAAGAGGCTGCAGACGTTAATGAG
```

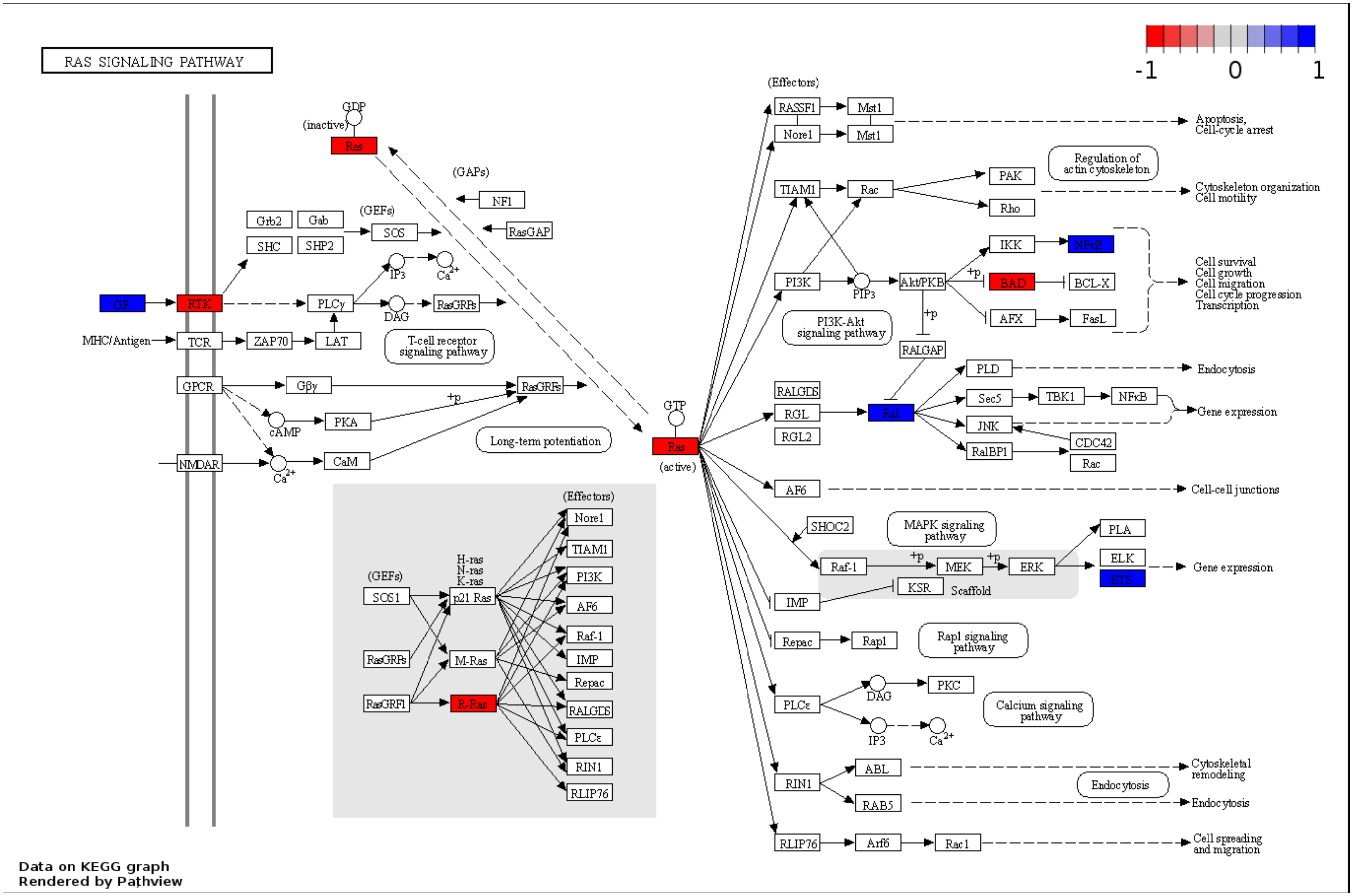
```
>MEG3-236_ENST00000648820_Exon6
```

```
GGTGTCCCAGCACCAGCCTGGCACAGAGTGGG
```

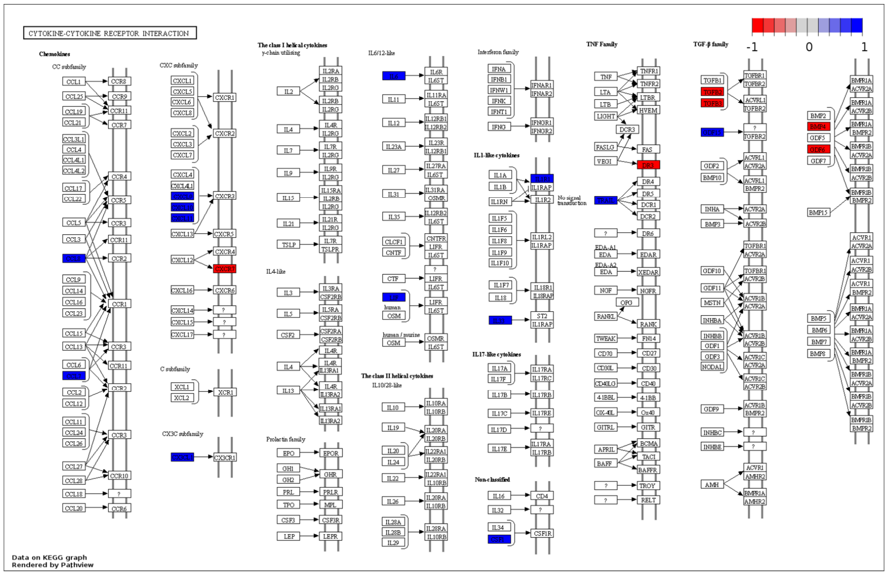
Supplementary Figure 1. MEG3 ENST00000398461 and ENST00000648820 transcript comparison. DE MEG3 transcripts were aligned to understand the biological meaning of their opposed expression level and the probability of having different roles during astrocytic lipotoxicity. (A) Graphic representation of MEG3 gene sequence alignment against ENST00000398461 (also known as MEG3-202, in blue) and ENST00000398461 (or MEG3-236, in green) transcripts. (B) Zoom into the overlapping region. (C) MEG3 ENST00000398461 transcript exons. (D) MEG3 ENST00000648820 transcript exons. The overlapping region is shown in a black square.

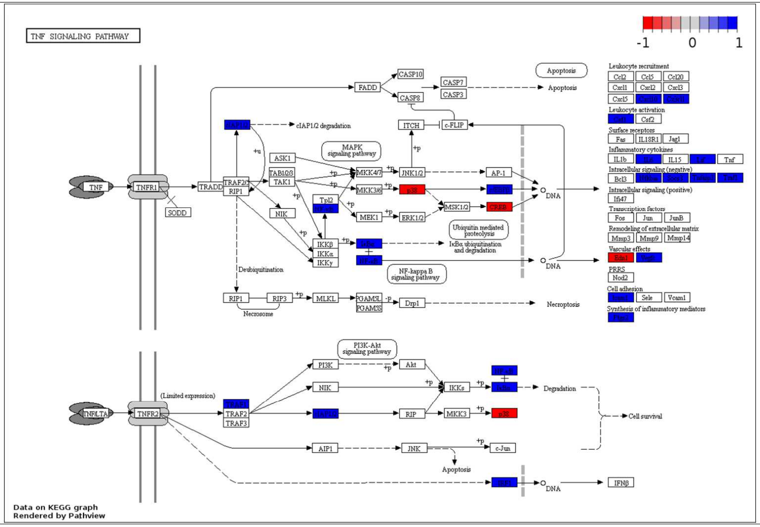

P53 SIGNALING PATHWAY
-1
0
1
Response
γ-irradiation
UV
Genotoxic drugs
Nutrition deprivation
Heat/cold shock
Stress signals
DNA damage
ATM
ATR
CHK2
CHK1
+p
Hypoxia
Nitric oxide
Oncogene activation (Such as MYC, E2F1, Ras, BCR-ABL)
SCYL1BP1
p14 ARF
MDM2
MDM-X
p53
DNA
Cell cycle
Target genes
p21
14-3-3-σ
Reprimo
Gadd45
B99
Fas
PIDD
Bax
Sival
PIGs
Scotin
PERP
PAG608
Siah
AIFM2
IGF-BP3
IGF
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
P48
p53R2
Gadd45
Sestrins
PTEN
TSC2
IGF-BP3
TSAP6
MDM2
Cop-1
PIRH-2
Cyclin G
Siah-1
Wip1
ΔNp73
Cyclin D
CDK4/6
Cyclin E
CDK2
Cyclin B
Cdc2
G1 arrest (sustained)
G2 arrest (sustained)
Cell cycle arrest
Cell cycle
Cellular senescence
DR5
CASP8
Bid
tBid
Noxa
PUMA
P53AIP
Bcl-xL
Bcl2
ROS
Mitochondrion
CytC
Apaf-1
CASP9
CASP3
Apoptosis
Apoptosis
Inhibition of angiogenesis and metastasis
DNA repair and damage prevention
Inhibition of IGF-1/mTOR pathway
Exosome mediated secretion
p53 negative feedback
Data on KEGG graph
Rendered by Pathview

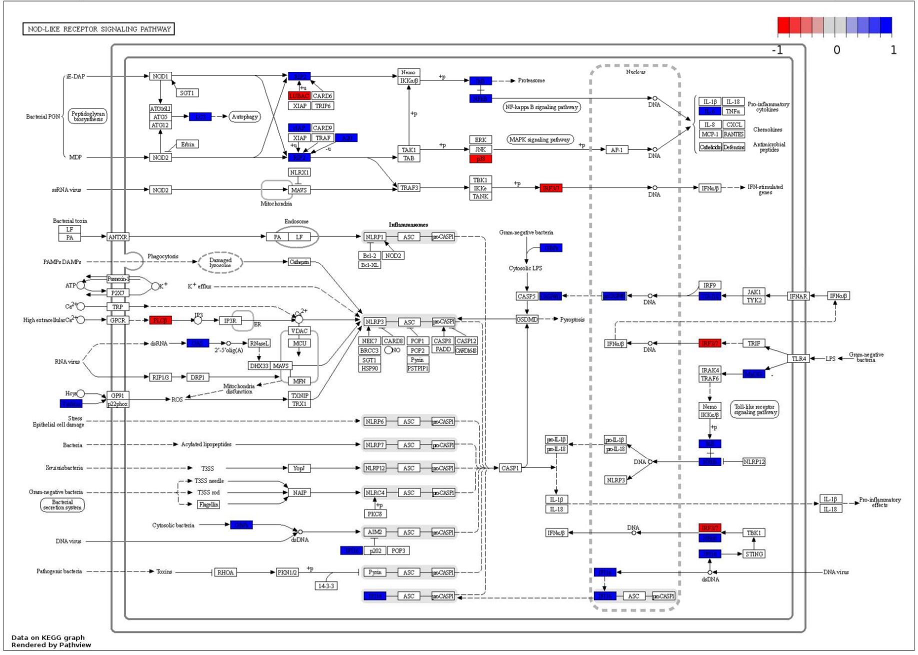
Supplementary Figure 2. p53 signaling pathway enrichment. DE genes in the transcriptome are displayed in the pathway according to their Log_2 Fold change, with dark blue for higher values and dark red for lower values.


RAS SIGNALING PATHWAY
-1
0
1
GF
RTK
MHC/Antigen
TCR
ZAP70
LAT
GPCR
Gβγ
NMDAR
Grb2
Gab
SHC
SHP2
(GEFs)
SOS
PLCγ
IP3
Ca2+
DAG
RasGRFs
T-cell receptor signaling pathway
cAMP
PKA
CaM
+p
RasGRFs
Long-term potentiation
GDP
(inactive)
Ras
(GAPs)
NF1
RasGAP
GTP
Ras
(active)
(Effectors)
(GEFs)
SOS1
RasGRFs
RasGRFl
H-ras
N-ras
K-ras
p21 Ras
M-Ras
R-Ras
Nore1
TIAM1
PI3K
AF6
Raf-1
IMP
Repac
RALGDS
PLCε
RIN1
RLIP76
(Effectors)
RASSF1
Nore1
Mst1
Mst1
Apoptosis, Cell-cycle arrest
TIAM1
Rac
PAK
Rho
Regulation of actin cytoskeleton
Cytoskeleton organization
Cell motility
PI3K
PIP3
Akt/PKB
IKK
NFκB
BAD
BCL-X
AFX
FasL
+p
Cell survival
Cell growth
Cell migration
Cell cycle progression
Transcription
PI3K-Akt signaling pathway
RALGAP
RALGDS
RGL
RGL2
Ral
PLD
Endocytosis
Sec5
TBK1
NFκB
Gene expression
JNK
CDC42
RalBP1
Rac
AF6
Cell-cell junctions
SHOC2
MAPK signaling pathway
Raf-1
MEK
ERK
KSR
Scaffold
PLA
ELK
ETS
Gene expression
IMP
Repac
Rap1
Rap1 signaling pathway
PLCε
DAG
PKC
IP3
Ca2+
Calcium signaling pathway
RIN1
ABL
Cytoskeletal remodeling
Endocytosis
RAB5
Endocytosis
RLIP76
Arf6
Rac1
Cell spreading and migration
Data on KEGG graph
Rendered by Pathview

Supplementary Figure 3. Ras signaling pathway enrichment. DE genes in the transcriptome are displayed in the pathway according to their Log_2 Fold change, with dark blue for higher values and dark red for lower values.


CYTOKINE-CYTOKINE RECEPTOR INTERACTION
-1
0
1
Chemokines
CC subfamily
CXC subfamily
C subfamily
CX3C subfamily
The class I helical cytokines
γ-chain utilizing
IL4-like
Prolactin family
IL6/12-like
The class II helical cytokines
IL10/28-like
Interferon family
IL1-like cytokines
IL17-like cytokines
Non-classified
TNF Family
TGF-β family
Data on KEGG graph
Rendered by Pathview


TNF SIGNALING PATHWAY
-1
0
1
TNF
TNFR1
TRADD
TRAF2/5
RIP1
FADD
CASP10
CASP8
CASP7
CASP3
Apoptosis
cIAP1/2 degradation
MAPK signaling pathway
ITCH
c-FLIP
ASK1
TAB1/2/3
TAK1
MKK4/7
MKK3/6
JNK1/2
p38
AP-1
MSK1/2
CREB
NIK
IKKβ
IKKα
IKKγ
NF-kappa B signaling pathway
IκBα ubiquitination and degradation
Ubiquitin mediated proteolysis
MEK1
ERK1/2
Tpl2
DNA
Deubiquitination
RIP1
RIP3
MLKL
PGAM5L
PGAM5S
Drp1
Necroptosis
Necrosome
SODD
Leukocyte recruitment
Leukocyte activation
Surface receptors
Inflammatory cytokines
Intracellular signaling (negative)
Intracellular signaling (positive)
Transcription factors
Remodeling of extracellular matrix
Vascular effects
PRRS
Cell adhesion
Synthesis of inflammatory mediators
TNF/LTA
TNFR2
(Limited expression)
TRAF2
TRAF3
PI3K-Akt signaling pathway
PI3K
Akt
IKKs
NIK
RIP
MKK3
p38
AIP1
JNK
c-Jun
Degradation
Cell survival
IFNβ
Data on KEGG graph
Rendered by Pathview


NOD-LIKE RECEPTOR SIGNALING PATHWAY
-1
0
1
iE-DAP
Bacterial PGN
Peptidoglycan biosynthesis
MDP
ssRNA virus
NOD1
NOD2
SGT1
Autophagy
Erbin
Nucleus
NF-kappa B signaling pathway
MAPK signaling pathway
Proteasome
AP-1
DNA
Pro-inflammatory cytokines
Chemokines
Antimicrobial peptides
IFN-stimulated genes
Mitochondria
Bacterial toxin
Endosome
Inflammasomes
Phagocytosis
Damaged lysosome
PAMPs DAMPs
K+ efflux
High extracellular Ca2+
dsRNA
RNA virus
Mitochondria dysfunction
ROS
Stress
Epithelial cell damage
Bacteria
Acylated lipopeptides
Yersinia bacteria
Gram-negative bacteria
Bacterial secretion system
Cytosolic bacteria
DNA virus
Pathogenic bacteria
Toxins
Gram-negative bacteria
Cytosolic LPS
Pyroptosis
Toll-like receptor signaling pathway
LPS
DNA virus
Pro-inflammatory effects
Data on KEGG graph
Rendered by Pathview

Supplementary Figure 4. Inflammatory pathways enrichment. DE genes in the transcriptome are displayed in the pathway according to their Log_2 Fold change, with dark blue for higher values and dark red for lower values.

1.2 Supplementary Table Legends

Supplementary Table 1. Primers. Primers used for validation of the selected axis are listed here.

Supplementary Table 2. DE genes in the transcriptome. Sheet tab "PA-DEG_up" displays all the significantly upregulated genes in the transcriptome, while sheet tab "PA-DEG_down" displays all the significantly downregulated genes in the transcriptome. Comparisons were made between PA-treated astrocytes (PA) and vehicle astrocytes (VH). Gene ID, symbol, biotype, Log_2 Fold change, p-value, and FDR-corrected p-value are shown for every gene.

Supplementary Table 3. Complete list of Panther enriched terms. Sheet tabs "MIR22HG (ENST00000334146)", "SDCBP2-AS1 (ENST00000446423)", and "MEG3 (ENST00000398461)" display the whole list of Panther enriched terms from the mRNAs related to MIR22HG (ENST00000334146), SDCBP2-AS1 (ENST00000446423), and MEG3 (ENST00000398461), respectively. Sheet tab "lncRNAs_up" shows the list of Panther enriched terms from the mRNAs related to the positively regulated lncRNAs, while sheet tab "lncRNAs_down" shows the whole list of Panther enriched terms from the mRNAs related to the negatively regulated lncRNAs.

Supplementary Table 4. Complete list of miRPath enriched KEGG pathways. Each sheet tab displays the whole list of enriched terms obtained from the miRNAs that interact with MEG3 (ENST00000396181), MEG3 (ENST00000648820), LINC01503 (ENST00000444125), AC092687.3 (ENST00000606907), SERTAD4-AS1 (ENST00000437764), SDCBP2-AS1 (ENST00000446423), and MIR22HG (ENST00000334146) lncRNAs, respectively. FDR-corrected p-value and the number of related genes and miRNAs are shown for each pathway.

Supplementary Table 5. Biological role of each RNA molecule in the selected axes, according to databases. Relative expression obtained from BioGPS and CNS microRNA profiles is presented as "above," "on," or "under" the median expression of total tissues in the databases.

Supplementary Table 6. Trimmed mean of M-values (TMM) of developmental and functional genes in mature astrocytes. The TMM normalized expression of *ALDH18A1*, *SOX9*, *GFAP*, *GJB2*, and *SLC1A3* genes is displayed for the replicates of AstroPA (PA-treated astrocytes) and AstroVH (VH astrocytes).