

# **Evaluating the Therapeutic Potential of Genetically Engineered Probiotic Zbiotics (ZB183) for Non-Alcoholic Steatohepatitis (NASH) Management via Modulation of the cGAS-STING Pathway**

## **Supplementary Material**

### **Supplementary Tables:**

#### **S1. Table: List of primers used for RT-PCR:**

<b>Primer Assay</b>	<b>GeneGlobe ID</b>
TNF (NM_012675)	QT00178717
MAPK3 (NM_017347)	QT00176330
EDN1 (NM_012548)	QT00371308
GAPDH (NM_001256799)	QT00079247
Hs_miR-6888-5p_1	MS00048069
Hs_SNORD72_11	MS00033719
RABGAP1L-IT1 (ENST00000414890)	SBH0671985

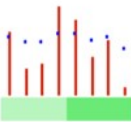
### **Supplementary Figures:**

**S1 Figures:** Validation of the relation between EDN1, *MAPK3*, & TNF, genes to NAFLD/NASH pathogenesis, B cell proliferation/Cytokine response by public microarray databases


EDN1 expression in NAFLD/NASH

- [Edn1 - Monoacylglycerol acyltransferase 1 deficiency effect on high-fat diet fed mice: liver](#)
  1. [mice: liver](#)

Annotation: **Edn1**, endothelin 1  
Organism: Mus musculus  
Reporter: GPL6887, ILMN\_2737713 (ID\_REF), GDS5425, NM\_010104, **EDN1** (ORF)  
DataSet type: Expression profiling by array, count, 8 samples  
ID: 125816065  
[GEO DataSets](#) [Gene](#) [Profile neighbors](#) [Chromosome neighbors](#) [Homologene neighbors](#)


- [Edn1 - Non-alcoholic steatohepatitis model](#)
  2. [Non-alcoholic steatohepatitis model](#)

Annotation: **Edn1**, endothelin 1  
Organism: Rattus norvegicus  
Reporter: GPL85, M64711\_at (ID\_REF), GDS3431, 24323 (Gene ID), M64711  
DataSet type: Expression profiling by array, count, 6 samples  
ID: 56830594  
[GEO DataSets](#) [Gene](#) [Profile neighbors](#) [Chromosome neighbors](#) [Homologene neighbors](#)


- [Edn1 - Liver response to a metformin supplemented high fat diet deficient in methionine and choline](#)
  3. [Liver response to a metformin supplemented high fat diet deficient in methionine and choline](#)

Annotation: **Edn1**, endothelin 1  
Organism: Mus musculus  
Reporter: GPL1261, 1451924\_a\_at (ID\_REF), GDS4883, 13614 (Gene ID), D43775  
DataSet type: Expression profiling by array, count, 12 samples  
ID: 106318119  
[GEO DataSets](#) [Gene](#) [Profile neighbors](#) [Chromosome neighbors](#) [Homologene neighbors](#)


- [EDN1 - Postbariatric, morbidly obese patients with nonalcoholic fatty liver disease: liver biopsies](#)
  4. [Postbariatric, morbidly obese patients with nonalcoholic fatty liver disease: liver biopsies](#)

Annotation: **EDN1**, endothelin 1  
Organism: Homo sapiens  
Reporter: GPL11532, 8116921 (ID\_REF), GDS4881, NM\_001955, NM\_001168319, BC009720, chr6:12290596-12297427 (SPOT ID)  
DataSet type: Expression profiling by array, transformed count, 73 samples  
ID: 106211796  
[GEO DataSets](#) [Gene](#) [Chromosome neighbors](#) [Homologene neighbors](#)



EDN1 Gene ontology

https://www.ebi.ac.uk/gxa/genes/ensg00000078401?bs="["homo sapiens"%3A"TC ... Search

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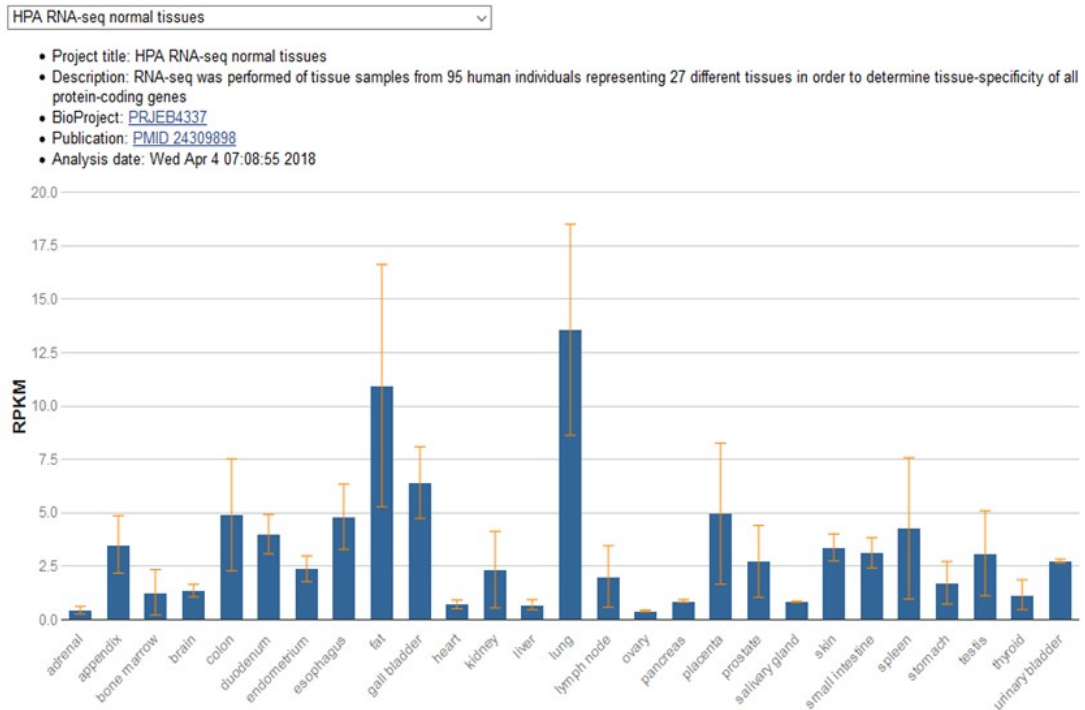
domestica], EDN1 (Canis familiaris), EDN1 (Gallus gallus), EDN1 (Anas platyrhynchos), EDN1 (Pongo abelii), EDN1 (Macaca mulatta)

positive regulation of JUN kinase activity, positive regulation of cytosolic calcium ion concentration involved in phospholipase C-activating G-protein coupled signaling pathway, negative regulation of smooth muscle cell apoptotic process, vein smooth muscle contraction, artery smooth muscle contraction, positive regulation of cytosolic calcium ion concentration, positive regulation of MAP kinase activity, vasoconstriction, positive regulation of transcription by RNA polymerase II, regulation of pH, negative regulation of transcription by RNA polymerase II, phosphatidylinositol 3-kinase signaling, positive regulation of NIK/NF-kappaB signaling, positive regulation of DNA-binding transcription factor activity, Weibel-Palade body, negative regulation of nitric-oxide synthase biosynthetic process, regulation of sensory perception of pain, adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway, positive regulation of prostaglandin secretor, positive regulation of chemokine-mediated signaling pathway, cellular response to glucocorticoid stimulus, cellular response to mineralocorticoid stimulus, positive regulation of mitotic nuclear division, positive regulation of neutrophil chemotaxis, endothelin A receptor binding, endothelin B receptor binding, prostaglandin biosynthetic process, positive regulation of vascular smooth muscle cell proliferation, rhythmic excitation, in utero embryonic development, regulation of systemic arterial blood pressure by endothelin, regulation of glucose transmembrane transport, inositol phosphate-mediated signaling, positive regulation of cell growth involved in cardiac muscle cell development, protein kinase C deactivation, positive regulation of smooth muscle cell proliferation, cellular response to calcium ion, positive regulation of endothelial cell migration, positive regulation of receptor biosynthetic process, negative regulation of cellular protein metabolic process, regulation of vasoconstriction, positive regulation of cardiac muscle hypertrophy, calcium-mediated signaling, negative regulation of gene expression, positive regulation of cell migration, positive regulation of prostaglandin-endoperoxide synthase activity, positive regulation of smooth muscle contraction, cellular response to tumor necrosis factor, positive regulation of sarcomere organization, cellular response to interleukin-1, cellular response to interferon-gamma, rough endoplasmic reticulum lumen, histamine secretion, cellular response to peptide hormone stimulus, sensory perception of pain, phospholipase D-activating G-protein coupled receptor signaling pathway, transport vesicle, response to prostaglandin F, positive regulation of nitric oxide biosynthetic process, positive regulation of cell proliferation, superoxide anion generation, positive regulation of cell size, cellular response to fatty acid, positive regulation of urine volume, endothelin receptor signaling pathway, positive regulation of hormone secretion, epithelial fluid transport, negative regulation of hormone secretion, response to dexamethasone, positive regulation of heart rate, peptide hormone secretion, negative regulation of blood coagulation, neural crest cell development, nitric oxide transport, cellular response to transforming growth factor beta stimulus, branching involved in blood vessel morphogenesis, positive regulation of odontogenesis, protein kinase C-activating G-protein coupled receptor signaling pathway, blood vessel morphogenesis, dorsal/ventral pattern

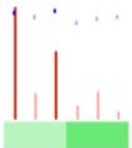
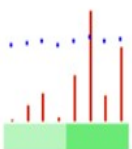
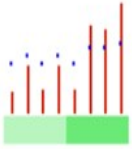
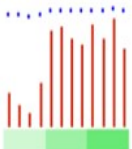
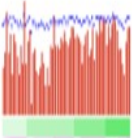
Gene ontology

Feedback

EDN1 expression in different tissues especially liver



MAPK3 expression in NAFLD/NASH

- [Mapk3 - Non-alcoholic steatohepatitis model](#)
  1. Annotation: **Mapk3**, mitogen activated protein kinase 3  
 Organism: Rattus norvegicus  
 Reporter: GPL85, M61177\_s\_at (ID\_REF), GDS3431, 50689 (Gene ID), M61177  
 DataSet type: Expression profiling by array, count, 6 samples  
 ID: 56830541  
[GEO DataSets](#)   [Gene](#)   [Profile neighbors](#)   [Chromosome neighbors](#)   [Homologene neighbors](#)
  
- [Mapk3 - Monoacylglycerol acyltransferase 1 deficiency effect on high-fat diet fed mice: liver](#)
  2. Annotation: **Mapk3**, mitogen-activated protein kinase 3  
 Organism: Mus musculus  
 Reporter: GPL6887, ILMN\_1218551 (ID\_REF), GDS5425, NM\_011952, **MAPK3** (ORF)  
 DataSet type: Expression profiling by array, count, 8 samples  
 ID: 125772794  
[GEO DataSets](#)   [Gene](#)   [Profile neighbors](#)   [Chromosome neighbors](#)   [Homologene neighbors](#)
  
- [Mapk3 - Monoacylglycerol acyltransferase 1 deficiency effect on high-fat diet fed mice: liver](#)
  3. Annotation: **Mapk3**, mitogen-activated protein kinase 3  
 Organism: Mus musculus  
 Reporter: GPL6887, ILMN\_2715744 (ID\_REF), GDS5425, NM\_011952, **MAPK3** (ORF)  
 DataSet type: Expression profiling by array, count, 8 samples  
 ID: 125810648  
[GEO DataSets](#)   [Gene](#)   [Profile neighbors](#)   [Chromosome neighbors](#)   [Homologene neighbors](#)
  
- [Mapk3 - Liver response to a metformin supplemented high fat diet deficient in methionine and choline](#)
  4. Annotation: **Mapk3**, mitogen-activated protein kinase 3  
 Organism: Mus musculus  
 Reporter: GPL1261, 1427060\_at (ID\_REF), GDS4883, 26417 (Gene ID), BI414398  
 DataSet type: Expression profiling by array, count, 12 samples  
 ID: 106293266  
[GEO DataSets](#)   [Gene](#)   [Profile neighbors](#)   [Chromosome neighbors](#)   [Homologene neighbors](#)
  
- [MAPK3 - Postbariatric, morbidly obese patients with nonalcoholic fatty liver disease: liver biopsies](#)
  5. Annotation: **MAPK3**, mitogen-activated protein kinase 3  
 Organism: Homo sapiens  
 Reporter: GPL11532, 8000811 (ID\_REF), GDS4881, NM\_001040056, NM\_002746,



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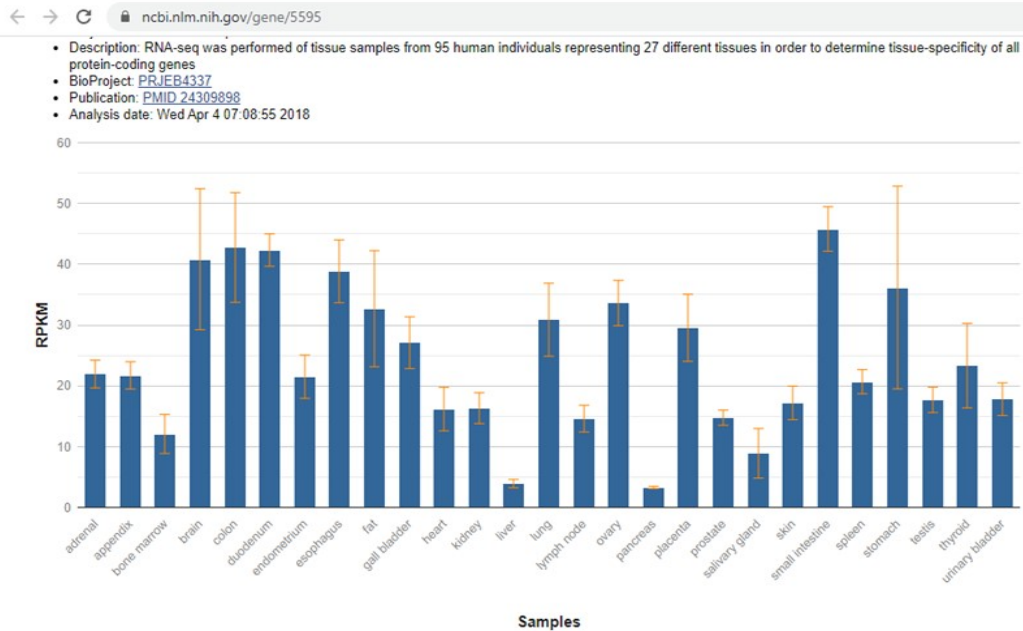
aries), MAPK3 (Pongo abelii), MAPK3 (Oryctolagus cuniculus), MAPK3 (Sus scrofa), Mapk3 (Mus musculus), MAPK3 (Macaca mulatta), MAPK3 (Pan troglodytes), MAPK3 (Equus caballus), MAPK3 (Bos taurus), MAPK3 (Gorilla gorilla)

activation of MAPK activity, activation of MAPKK activity, positive regulation of histone phosphorylation, positive regulation of histone acetylation, stress-activated MAPK cascade, positive regulation of transcription by RNA polymerase II, regulation of cellular pH, peptidyl-tyrosine autophosphorylation, positive regulation of protein phosphorylation, ERK1 and ERK2 cascade, positive regulation of ERK1 and ERK2 cascade, regulation of Golgi inheritance, Fc-epsilon receptor signaling pathway, Fc-gamma receptor signaling pathway involved in phagocytosis, MAPK cascade, positive regulation of cytokine secretion involved in immune response, positive regulation of metalloproteinase activity, positive regulation of translation, transcription initiation from RNA polymerase I promoter, fibroblast growth factor receptor signaling pathway, BMP signaling pathway, peptidyl-serine phosphorylation, regulation of phosphatidylinositol 3-kinase signaling, regulation of DNA-binding transcription factor activity, caveolin-mediated endocytosis, DNA damage induced protein phosphorylation, regulation of cytoskeleton organization, Bergmann glial cell differentiation, positive regulation of telomere capping, negative regulation of apolipoprotein binding, positive regulation of macrophage chemotaxis, cellular response to cadmium ion, positive regulation of xenophagy, positive regulation of telomerase activity, positive regulation of gene expression, regulation of stress-activated MAPK cascade, late endosome, protein phosphorylation, early endosome, cellular response to tumor necrosis factor, transcription, DNA-templated, cardiac neural crest cell development involved in heart development, positive regulation of telomere maintenance via telomerase, interleukin-1-mediated signaling pathway, cellular response to amino acid starvation, phosphotyrosine residue binding, arachidonic acid metabolic process, MAP kinase kinase activity, MAP kinase activity, sensory perception of pain, cellular response to reactive oxygen species, regulation of early endosome to late endosome transport, regulation of cellular response to heat, thymus development, thyroid gland development, positive regulation of cyclase activity, neural crest cell development, regulation of gene expression, lipopolysaccharide-mediated signaling pathway, ATP binding, response to exogenous dsRNA, phosphorylation, cellular response to dopamine, axon guidance, caveola, intracellular signal transduction, phosphatase binding, protein-containing complex assembly, cellular response to DNA damage stimulus, protein serine/threonine kinase activity, nuclear envelope, nucleus, lung morphogenesis, cellular response to organic substance, cellular response to mechanical stimulus, cytoskeleton, mitochondrion, response to lipopolysaccharide, trachea formation, Golgi apparatus, apoptotic process, cytosol, pseudopodium, cartilage development, outer ear morphogenesis, nucleoplasm, focal adhesion, viral process, cell surface receptor signaling pathway

Gene ontology

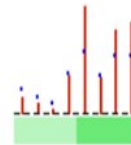
Feedback

MAPK3 expression in different tissues especially liver

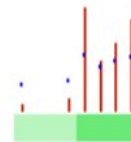


**TNF $\alpha$  expression in NAFLD/NASH**

[Tnf - Monoacylglycerol acyltransferase 1 deficiency effect on high-fat diet fed mice: liver](#)  
 1. [Tnf - Monoacylglycerol acyltransferase 1 deficiency effect on high-fat diet fed mice: liver](#)  
 Annotation: Tnf, tumor necrosis factor  
 Organism: Mus musculus  
 Reporter: GPL6887, ILMN\_2467245 (ID\_REF), GDS5425, NM\_013693, TNF (ORF)  
 DataSet type: Expression profiling by array, count, 8 samples  
 ID: 125816732  
[GEO DataSets](#) [Gene](#) [Profile neighbors](#) [Chromosome neighbors](#) [Homologene neighbors](#)



[Tnf - Monoacylglycerol acyltransferase 1 deficiency effect on high-fat diet fed mice: liver](#)  
 2. [Tnf - Monoacylglycerol acyltransferase 1 deficiency effect on high-fat diet fed mice: liver](#)  
 Annotation: Tnf, tumor necrosis factor  
 Organism: Mus musculus  
 Reporter: GPL6887, ILMN\_2899863 (ID\_REF), GDS5425, NM\_013693, TNF (ORF)  
 DataSet type: Expression profiling by array, count, 8 samples  
 ID: 125785899  
[GEO DataSets](#) [Gene](#) [Profile neighbors](#) [Chromosome neighbors](#) [Homologene neighbors](#)



[Tnf - Non-alcoholic steatohepatitis model](#)  
 3. [Tnf - Non-alcoholic steatohepatitis model](#)  
 Annotation: Tnf, tumor necrosis factor  
 Organism: Rattus norvegicus  
 Reporter: GPL85, L00981mRNA#2\_at (ID\_REF), GDS3431, 24835 (Gene ID), L00981  
 DataSet type: Expression profiling by array, count, 6 samples  
 ID: 56829638  
[GEO DataSets](#) [Gene](#) [Profile neighbors](#) [Chromosome neighbors](#) [Homologene neighbors](#)



[Tnf - Non-alcoholic steatohepatitis model](#)  
 4. [Tnf - Non-alcoholic steatohepatitis model](#)  
 Annotation: Tnf, tumor necrosis factor  
 Organism: Rattus norvegicus  
 Reporter: GPL85, E02468cds\_s\_at (ID\_REF), GDS3431, 24835 (Gene ID), E02468  
 DataSet type: Expression profiling by array, count, 6 samples  
 ID: 56829379  
[GEO DataSets](#) [Gene](#) [Profile neighbors](#) [Chromosome neighbors](#) [Homologene neighbors](#)



[Tnf - Liver response to a metformin supplemented high fat diet deficient in methionine and choline](#)  
 5. [Tnf - Liver response to a metformin supplemented high fat diet deficient in methionine and choline](#)  
 Annotation: Tnf, tumor necrosis factor  
 Organism: Mus musculus  
 Reporter: GPL1261, 1419607\_at (ID\_REF), GDS4883, 21926 (Gene ID), NM\_013693  
 DataSet type: Expression profiling by array, count, 12 samples

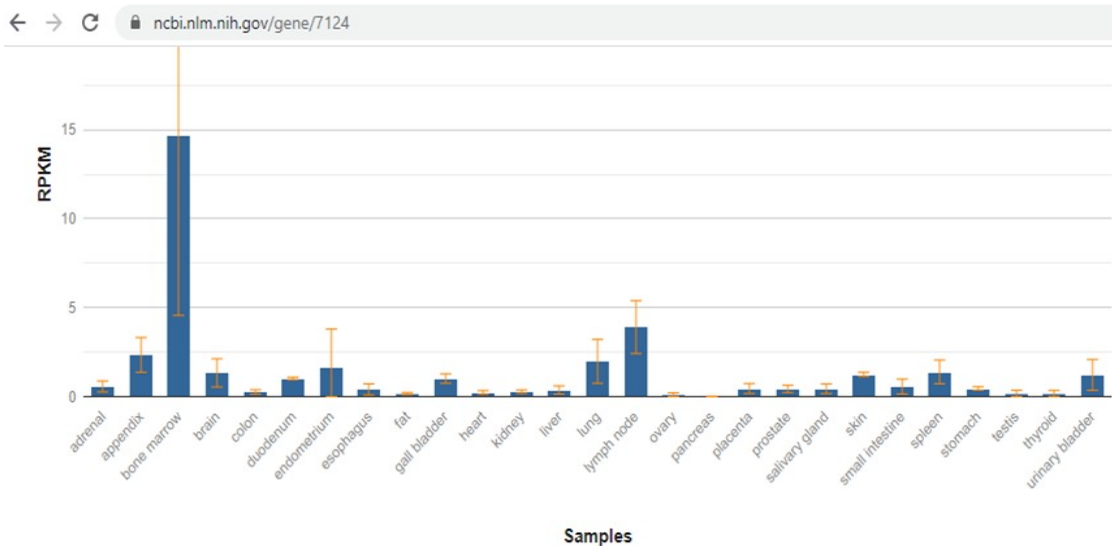


TNF $\alpha$  Gene ontology

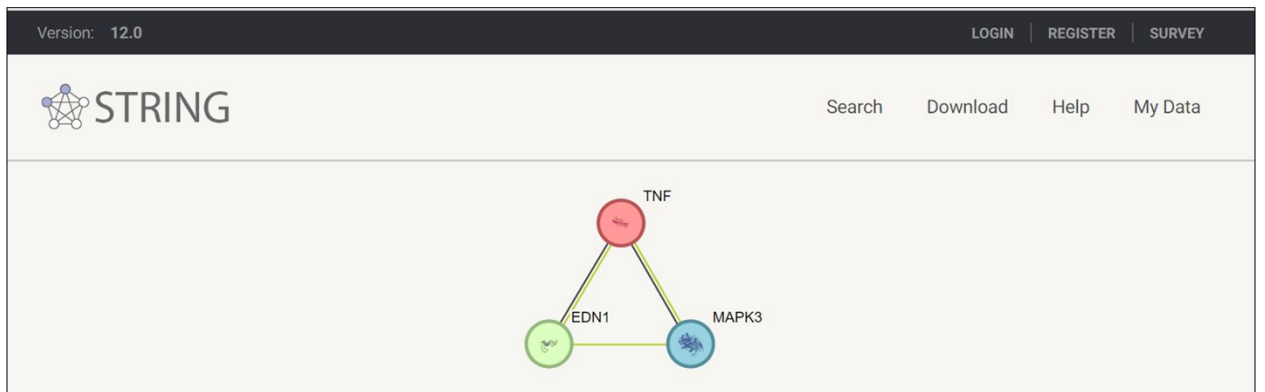
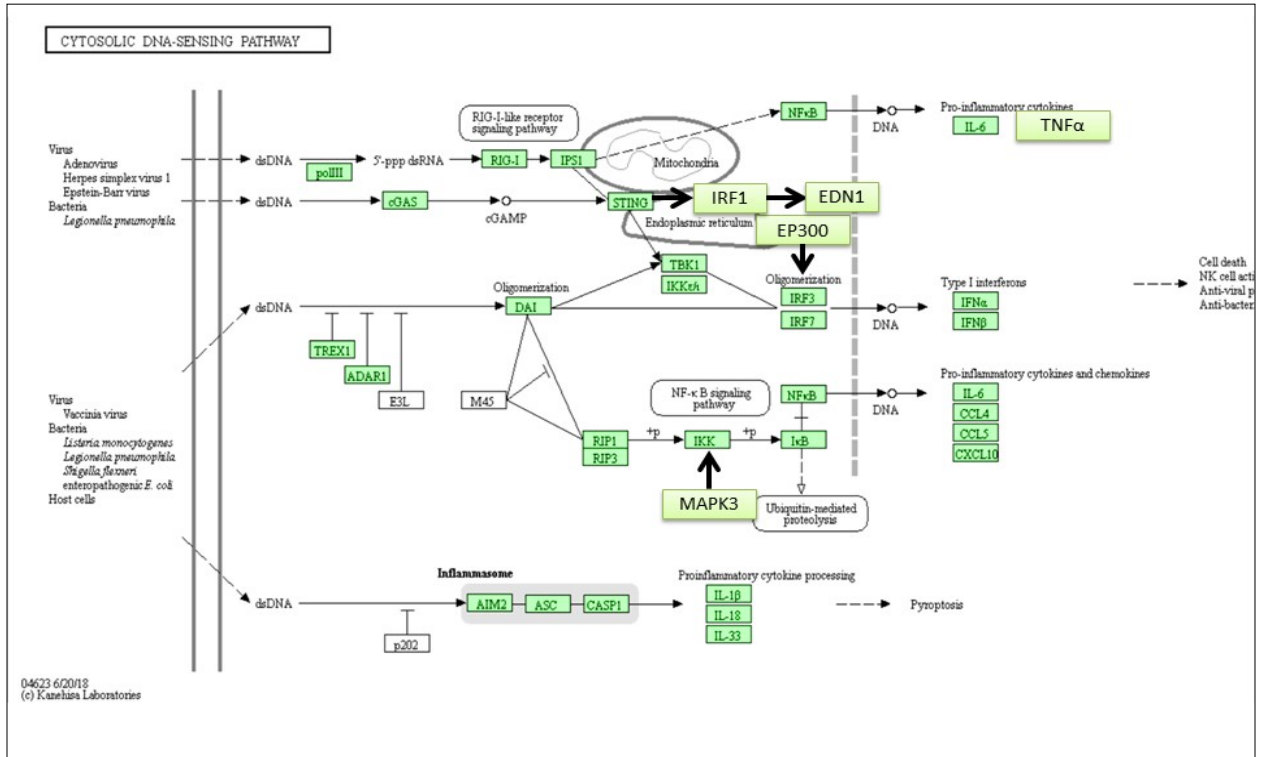
ENSACAG00000028461 (Anolis carolinensis), ENSCING00000008162 (Equus caballus), ENSTNIG00000000171 (Homo sapiens), TNF-alpha (Ovis aries), TNF (Pongo abelii)

positive regulation of JUN kinase activity, JNK cascade, positive regulation of tyrosine phosphorylation of STAT protein, activation of MAPK activity, activation of MAPKKK activity, positive regulation of peptidyl-serine phosphorylation, positive regulation of calcineurin-NFAT signaling cascade, activation of cysteine-type endopeptidase activity involved in apoptotic process, positive regulation of translational initiation by iron, positive regulation of NF-kappaB transcription factor activity, positive regulation of MAP kinase activity, positive regulation of leukocyte adhesion to arterial endothelial cell, positive regulation of transcription by RNA polymerase II, positive regulation of osteoclast differentiation, positive regulation of protein phosphorylation, positive regulation of fractalkine biosynthetic process, positive regulation of JAK-STAT cascade, positive regulation of cysteine-type endopeptidase activity involved in apoptotic process, negative regulation of myosin-light-chain-phosphatase activity, negative regulation of transcription by RNA polymerase II, negative regulation of alkaline phosphatase activity, regulation of endothelial cell apoptotic process, positive regulation of JNK cascade, positive regulation of ERK1 and ERK2 cascade, positive regulation of phosphatidylinositol 3-kinase signaling, positive regulation of NIK/NF-kappaB signaling, positive regulation of DNA-binding transcription factor activity, positive regulation of chronic inflammatory response to antigenic stimulus, positive regulation of nitric-oxide synthase activity, negative regulation of transcription, DNA-templated, positive regulation of transcription, DNA-templated, positive regulation of interleukin-6 biosynthetic process, positive regulation of interleukin-8 biosynthetic process, positive regulation of humoral immune response mediated by circulating immunoglobulin, positive regulation of protein kinase B signaling, regulation of establishment of endothelial barrier, positive regulation of I-kappaB kinase/NF-kappaB signaling, positive regulation of leukocyte adhesion to vascular endothelial cell, positive regulation of neuron apoptotic process, negative regulation of cytokine secretion involved in immune response, MAPK cascade, positive regulation of interleukin-6 secretion, regulation of osteoclast differentiation, negative regulation of extrinsic apoptotic signaling pathway in absence of ligand, regulation of protein phosphorylation, negative regulation of endothelial cell proliferation, positive regulation of membrane protein ectodomain proteolysis, positive regulation of caldiciol 1-monooxygenase activity, positive regulation of amyloid-beta formation, positive regulation of vitamin D biosynthetic process, positive regulation of protein kinase activity, positive regulation of chemokine secretion, positive regulation of vascular smooth muscle cell proliferation, negative regulation of viral replication, regulation of glucose import, regulation of transcription by RNA polymerase II, positive regulation of apoptotic process.

TNF $\alpha$  expression in different tissues especially liver

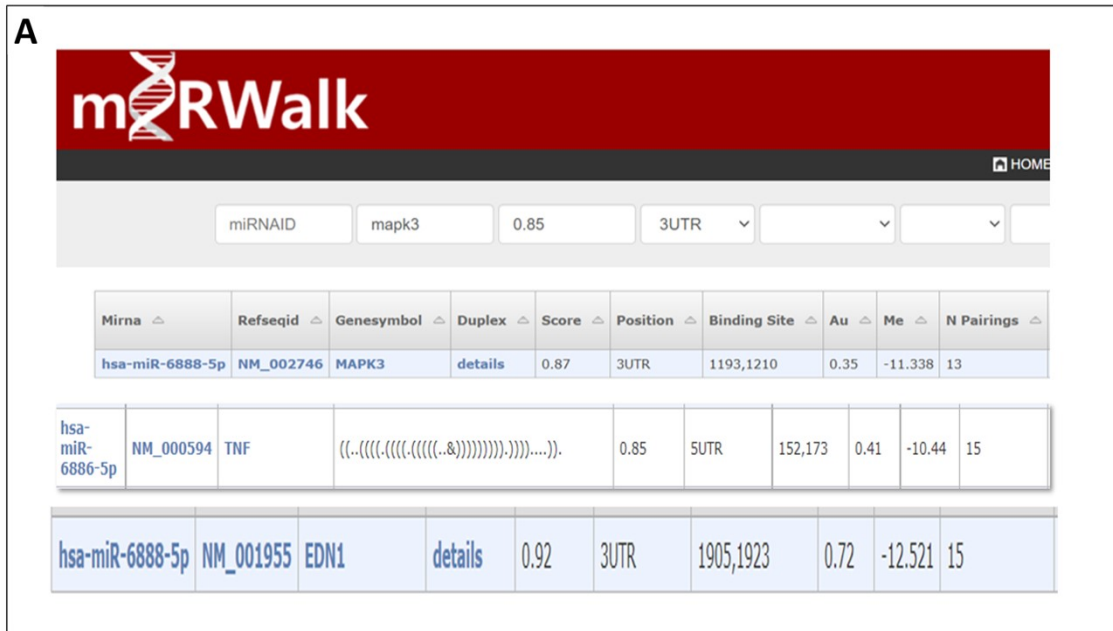


**S2 Figure:** Validation of the association of EDN1, MAPK3, & TNF with STING-mediated cytokine signaling in KEGG map and STRING-database





**S3 Figure: A)** Validation of the interaction between the selected mRNAs and the retrieved hsa-miR-6888-5p from the mirWalk database, **B)** Validation of the conservation of hsa-miR-6888-5p in wistar rats from the Blast database.



**B**

blast.ncbi.nlm.nih.gov/Blast.cgi

Your search is limited to records that include: *Rattus norvegicus* (taxid:10116)

Job Title: **hsa-miR-6888-5p**

RID: [E10JPNV013](#) Search expires on 09-12 01:31 am [Download All](#)

Program: BLASTN [Citation](#)

Database: refseq\_reference\_genomes (GPIPE/10116/RS\_2024\_02/ref\_top\_level) [See details](#)

Query ID: lcl|Query\_4674951

Description: None

Molecule type: nucleic acid

Query Length: 20

Other reports: [Distance tree of results](#) [MSA viewer](#)

**Filter Results**

Organism: only top 20 will appear  exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity:  to  E value:  to  Query Coverage:  to

[Filter](#) [Reset](#)

**Descriptions** Graphic Summary Alignments Taxonomy

**Sequences producing significant alignments** Download Select columns Show 100

select all 25 sequences selected

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc Len	Accession
<input checked="" type="checkbox"/> <a href="#">Rattus norvegicus strain BN/NHsdMcwi chromosome 10_GRCr8</a>	<i>Rattus norvegicus</i>	36.2	6228	100%	0.11	100.00%	107713808	NC_086028.1
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<input checked="" type="checkbox"/> <a href="#">Rattus norvegicus strain BN/NHsdMcwi chromosome 3_GRCr8</a>	<i>Rattus norvegicus</i>	34.2	7881	100%	0.44	100.00%	189428310	NC_086021.1
<input checked="" type="checkbox"/> <a href="#">Rattus norvegicus strain BN/NHsdMcwi chromosome 5_GRCr8</a>	<i>Rattus norvegicus</i>	32.2	7799	100%	1.7	100.00%	172190305	NC_086023.1
<input checked="" type="checkbox"/> <a href="#">Rattus norvegicus strain BN/NHsdMcwi chromosome 6_GRCr8</a>	<i>Rattus norvegicus</i>	32.2	6836	100%	1.7	100.00%	147156653	NC_086024.1

blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr\_2671914603

[Download](#) [GenBank](#) [Graphics](#) [Filter by: E value](#)

**Rattus norvegicus strain BN/NHsdMcwi chromosome 2\_GRCr8**

Sequence ID: **NC\_086020.1** Length: 251712708 Number of Matches: 365

Range 1: 158044305 to 158044321 GenBank Graphics

Score: 34.2 bits(1.7) Expect: 0.44 Identical: 17/17(100%) Gaps: 0/17(0%) Strand: Plus/Plus

Features: 1390514 bp at 5' side: otoln.1  
1700090 bp at 3' side: succinate:isomaltase\_intestinal

Query 2: AGAGATGCTAGGCAG 18  
Sbjct 158044305 ATTTTATTTTATTTTATTTT 158044321

Range 2: 16129942 to 16129957 GenBank Graphics

Score: 32.2 bits(1.6) Expect: 1.7 Identical: 16/16(100%) Gaps: 0/16(0%) Strand: Plus/Minus

Features: 197100 bp at 5' side: uncharacterized protein DRD9.2HG isoform x1  
251403 bp at 3' side: uncharacterized protein 161b isoform x4

Query 4: GAGATGCTAGGCAGA 19  
Sbjct 16129942 ATTTTATTTTATTTTATTTT 16129942

**Related Information**  
Genome Data Browser  
genomic context

**S4 Figure:** Validation of the interaction between the retrieved hsa-miR-6888-5p and lncRNA RABGAP1L-DT-206

miRNA	SPMS	LncRNA(GeneID)	LncRNA (TransID)	LncRNA (Transcript)	LncRNA	SL	SeedS	SeedE	pvalue
<a href="#">hsa-miR-6888-5p</a>	1	<a href="#">ENSG00000227373</a>	<a href="#">ENST00000454467</a>	<a href="#">RP11-160H22.5-003</a>	<a href="#">RP11-160H22.5</a>	11	226	216	0

CLUSTAL O(1.2.4) multiple sequence alignment

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test2 GTAGCAGCGGGCGGCAGCCGCCGAGGCAGCAATGTCAGGAGGGCACAGCTGGAGGAGGCG

test1 -----CCCGCAGG-----
test2 GCCGCCAAAGCCACTACCCACCCCGAGGAAGATTTGGAAAAGAGGCAC TGGCTACTGGA
      *** **

test1 -----
test2 GGAAGGGAGAAATCACCGAGAGAGCGAAAGATGTAACAGAAACAGGCTCTTGTTCTGTCA

test1 -----UGAGAUGAG-----
test2 CCCATACTTGGAGTGCAGTGGTACAATGATAGCTCACTGCAGCCTCAAAC TCCTGGTCTC
      * **

test1 -----
test2 AAGCAATCCTCTCACCTCAGCCTCCAGATGCAGTGACTCATGCCTATAATCCAGTACT

test1 -----
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test1 -----
test2 TCCTGTAGTCCCAGCTACTCAAGAGGCTGATGTAGAGAATCGCATTATCCAGAGGTCAA

test1 GGCU-----
test2 GGCTGCAGTGAGCCGTGATCATGCCTCTGCAC TCCAGCCTGGACGATAGAGCAAG
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