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Βλάβη Ισχαιμίας/Επαναιμάτωσης του Αμφιβληστροειδούς***

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***In Silico and In Vivo Investigation of Taurine's Effects
on Retinal Ischemia/Reperfusion Injury***

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ΕΥΧΑΡΙΣΤΙΕΣ

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ΠΕΡΙΛΗΨΗ

Η ταυρίνη έχει επιδείξει ευεργετικές δράσεις σε πληθώρα ασθενειών, ωστόσο ο ρόλος της στην αμφιβληστροειδική βλάβη ισχαιμίας/επαναιμάτωσης (RIRI, *Retinal Ischemia/Reperfusion Injury*) παραμένει σε μεγάλο βαθμό ασαφής. Η RIRI αποτελεί σημαντική αιτία διαταραχής ή και απώλειας της όρασης, κυρίως λόγω της έλλειψης αποτελεσματικών θεραπειών. Η Φαρμακολογία Δικτύων είναι πολύτιμη για τη φαρμακολογική στόχευση πολύπλοκων παθολογιών, όπως η RIRI, όπου η κατανόηση των αλληλεπιδράσεων μεταξύ των βιολογικών μονοπατιών είναι απαραίτητη. Η παρούσα διατριβή είχε ως στόχο τη διερεύνηση των δράσεων της ταυρίνης στην RIRI τόσο *in silico* όσο και *in vivo*. Οι πιθανοί φαρμακολογικοί στόχοι της ταυρίνης συλλέχθηκαν με τη χρήση 9 βάσεων δεδομένων και διαδικτυακών εργαλείων, ενώ οι στόχοι που σχετίζονται με την βλάβη ισχαιμίας/επαναιμάτωσης (IRI) συλλέχθηκαν από 3 βάσεις δεδομένων. Το χρονοεξαρτώμενο γονιδιακό προφίλ της RIRI αρουραίου ανακτήθηκε από την [EMBL-EBI Gene Expression Atlas](#). Κατασκευάστηκαν τα δίκτυα αλληλεπίδρασης πρωτεΐνης-πρωτεΐνης των κοινών στόχων ταυρίνης-IRI και ταυρίνης-RIRI αρουραίου 24 ώρες και 7 ημέρες μετά την επαναιμάτωση του ιστού και προσδιορίστηκαν οι κεντρικοί στόχοι. Για τη *in silico* διερεύνηση των μηχανισμών δράσης της ταυρίνης πραγματοποιήθηκαν: (i) αναλύσεις εμπλουτισμού KEGG και GO για τους κοινούς στόχους, και (ii) προσομοιώσεις μοριακής πρόσδεσης για τους κεντρικούς στόχους. Για την *in vivo* μελέτη της δράσης της ταυρίνης κατά την RIRI, αρσενικοί αρουραίοι Wistar, ηλικίας 8 εβδομάδων, χωρίστηκαν τυχαία σε τέσσερις ομάδες. Δύο ομάδες έλαβαν 0,2M ταυρίνης στο πόσιμο νερό για δύο εβδομάδες πριν από την RIRI και για 24 ώρες ή 7 ημέρες μετά την επαναιμάτωση του ιστού. Οι άλλες δύο ομάδες χρησίμευσαν ως αντίστοιχες ομάδες ελέγχου. Σε όλες τις ομάδες, προκλήθηκε στο ένα μάτι RIRI με αύξηση της ενδοφθάλμιας πίεσης στα 110 mmHg για 60 λεπτά, ενώ το άλλο μάτι χρησίμευσε ως ιστός μάρτυρας. Οι αμφιβληστροειδείς σημάνθηκαν ανοσοϊστοχημικά με τον δείκτη των αστροκυττάρων, όξινη πρωτεΐνη των ενδιάμεσων ινιδίων της γλοίας (GFAP) και έγινε μορφολογική αξιολόγηση της ενεργοποίησης των αστροκυττάρων. Σύμφωνα με την ανάλυση εμπλουτισμού, οι κοινοί στόχοι ταυρίνης-IRI εντοπίζονται κυρίως σε εξωκυτταρικά κυστίδια, σε κυστίδια κλαθρίνης, στις συνάψεις, σε σωμάτια έγκλεισης, και στη μήτρα των υπεροξειδωμάτων και σχετίζονται πρωτίστως με (i) βασικές βιολογικές διεργασίες όπως η απάντηση στα ερεθίσματα, ο μεταβολισμός, η ομοιοστάση και η ανοσολογική απόκριση, (ii) μοριακές λειτουργίες όπως η δέσμευση στις πρωτεΐνες ικρίωματος, η ενεργητική μεταφορά διαμέσου των μεμβρανών, ο αυτό-διμερισμός/πολυμερισμός των πρωτεϊνών και η φωσφορυλίωση μέσω PKC και (iii) σηματοδοτικά μονοπάτια όπως αυτά των λιπιδίων και αθηροσκλήρωσης, της απόπτωσης, της νευροεκφύλισης, του PI3K-Akt, του TNFα, του Ca⁺⁺, και της IL-17. Σύμφωνα με τις μελέτες προσομοίωσης μοριακού ελλιμενισμού η ταυρίνη φαίνεται να αλληλεπιδρά με τον EGFR. Σε σύγκριση με τα ζώα ελέγχου, οι αρουραίοι που έλαβαν ταυρίνη δεν παρουσίασαν σημαντική αύξηση των παραμέτρων των αστροκυττάρων, υποδεικνύοντας την προστατευτική δράση της ταυρίνης έναντι της αστρογλοίωσης που προκλήθηκε από RIRI. Τα ευρήματα αυτά υποδηλώνουν ότι η ταυρίνη ή ανάλογα αυτής θα μπορούσαν να αποτελέσουν στο μέλλον θεραπευτική επιλογή για την αντιμετώπιση της RIRI. Απαιτούνται περαιτέρω μελέτες για την επιβεβαίωση των αποτελεσμάτων μας και τη διαλεύκανση των μηχανισμών δράσης της ταυρίνης.

SUMMARY

Taurine has demonstrated therapeutic effects across various diseases, but its impact on retinal ischemia/reperfusion injury (RIRI) remains largely unexplored. RIRI is a major cause of visual impairment and blindness, primarily due to the lack of effective treatments. Network pharmacology is valuable for targeting complex pathologies like RIRI, as it facilitates the understanding of interactions among biological pathways essential for effective pharmacological intervention. This MSc thesis aimed to investigate the effects of taurine on RIRI both *in silico* and *in vivo*. Potential taurine targets were identified using 9 databases and webtools, while ischemia/reperfusion injury (IRI)-related targets were sourced from 3 databanks. A rat RIRI time-dependent gene profile was retrieved from the [EMBL-EBI Gene Expression Atlas](#). Protein-protein interaction (PPI) networks of shared taurine-IRI and taurine-RIRI targets at 24h and 7 days after reperfusion were constructed and core targets within the networks were identified. For the *in silico* investigation of taurine's mechanisms of action, (i) KEGG and GO enrichment analyses were performed on the common targets, and (ii) molecular docking simulations were conducted for the core targets. For the *in vivo* investigation of taurine effects during RIRI, 8-week-old male Wistar rats were randomly divided into four groups. Two groups received 0.2M taurine in drinking water for two weeks before RIRI and for 24 hours or 7 days after reperfusion. The other two groups served as the corresponding controls. In all animals, RIRI was induced in one eye by elevating intraocular pressure to 110mmHg for 60 minutes, with the other eye serving as a control. The retinas were immunohistochemically stained with the astrocytes marker, glial fibrillary acidic protein (GFAP) and morphological evaluation of astrocyte activation performed. According to enrichment analyses, the common taurine-IRI targets are mainly located in extracellular vesicles, clathrin-coated endocytic vesicles, synapses, inclusion bodies, and peroxisomal matrix and are primarily related to (i) biological processes such as stimulus response, metabolism, homeostasis, and immune response, (ii) molecular functions such as scaffold protein binding, active transmembrane transporter activity, protein self-association, PKC binding and hydrolase activity (iii) by signaling pathways such as lipid and atherosclerosis, apoptosis, neurodegeneration, PI3K-Akt, TNF α , Ca⁺⁺ and IL-17. Based on the molecular docking simulations, taurine directly interacts with the EGFR. Compared to control animals, rats receiving taurine showed no significant increase in astrocyte parameters, indicating a protective effect of taurine against RIRI-induced astrogliosis. These findings suggest that taurine, by targeting multiple pathways and reducing astrogliosis, may be a promising therapeutic option for RIRI treatment. Further studies are necessary to confirm these results and elucidate the underlying mechanisms.

1. INTRODUCTION

1.1. Taurine

Taurine (2-aminoethanesulfonic acid) is a naturally occurring semi-essential amino acid containing sulfur in the body^[1] ([Figure 1](#)). Taurine is the most abundant free amino acid in the nervous system and muscle tissue. Taurine concentrations in the human body can vary significantly depending on the tissue, ranging from 1 to 20 $\mu\text{mol/g}$ in the brain, 30 to 40 $\mu\text{mol/g}$ in the retina, and approximately 50 to 100 $\mu\text{mol/L}$ (μM) in plasma^[2]. Taurine is transported into the brain through the taurine transporter (TAUT), which is widely distributed in both the brain tissue and blood-brain barrier^[3]. Taurine is abundant in a diet rich in meat and seafood while it is also biosynthesized in the body from the amino acid cysteine^{[1],[4]}. Taurine's biosynthesis occurs mainly in the liver, retina, heart, and brain yet, it is important to notice that dietary intake is essential to fully meet the body's needs, particularly the ones of neurons.

In the brain, the synthesis of taurine occurs in astrocytes through three different pathways. The first and most prevalent pathway depends on cysteine as its main substrate. Cysteine is oxidized by cysteine dioxygenase to produce cysteine sulfinic acid (CAD), which is then converted to hypotaurine by cysteine sulfinic acid decarboxylase (CSAD). The second pathway uses extracellular methionine as its principal substrate. Methionine undergoes many enzymatic processes that lead to the production of cysteine, which is then metabolized following the first pathway. The third mechanism involves the breakdown of coenzyme-A, resulting in the production of cysteamine. Cysteamine is then converted into hypotaurine by the enzyme 2-aminoethanethiol dioxygenase. All three processes produce hypotaurine, which can either be converted into taurine and released from the cells or be released as hypotaurine and then converted to taurine in another cell, such as neurons^[2].

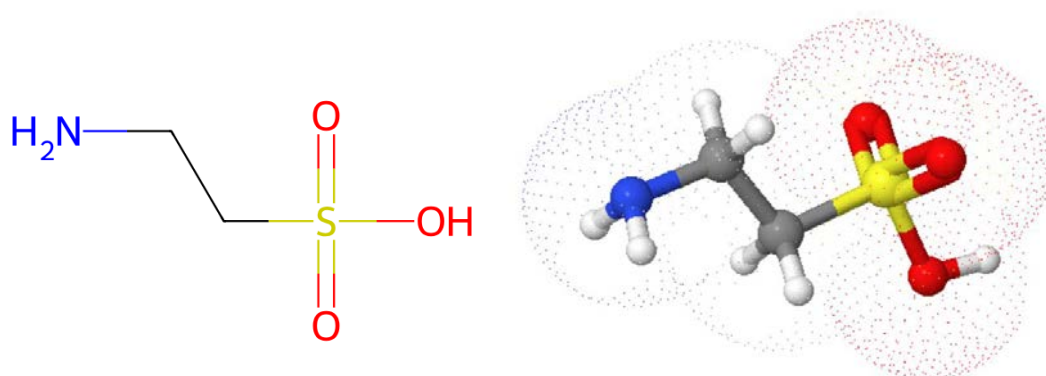


Figure 1. Chemical structure of Taurine 2D and 3D structure of taurine ([CHEMBL239243](#), [3DChem.com](#)).

Taurine's physiological function started showing up approximately 50 years ago. A study has shown that cats with low taurine plasma and retinal levels experience retinal degeneration, particularly at the photoreceptor level^[5]. Taurine's physiological role and its ability to protect against pathological events from diseases of the cardiovascular system, diabetes, metabolic syndrome, and nervous system have gained attention in recent years^{[6]-[12]}. Researchers have designed a model of diabetic nephropathy using taurine transporter (TauT) knockout animals resulting in taurine deficiency^[10].

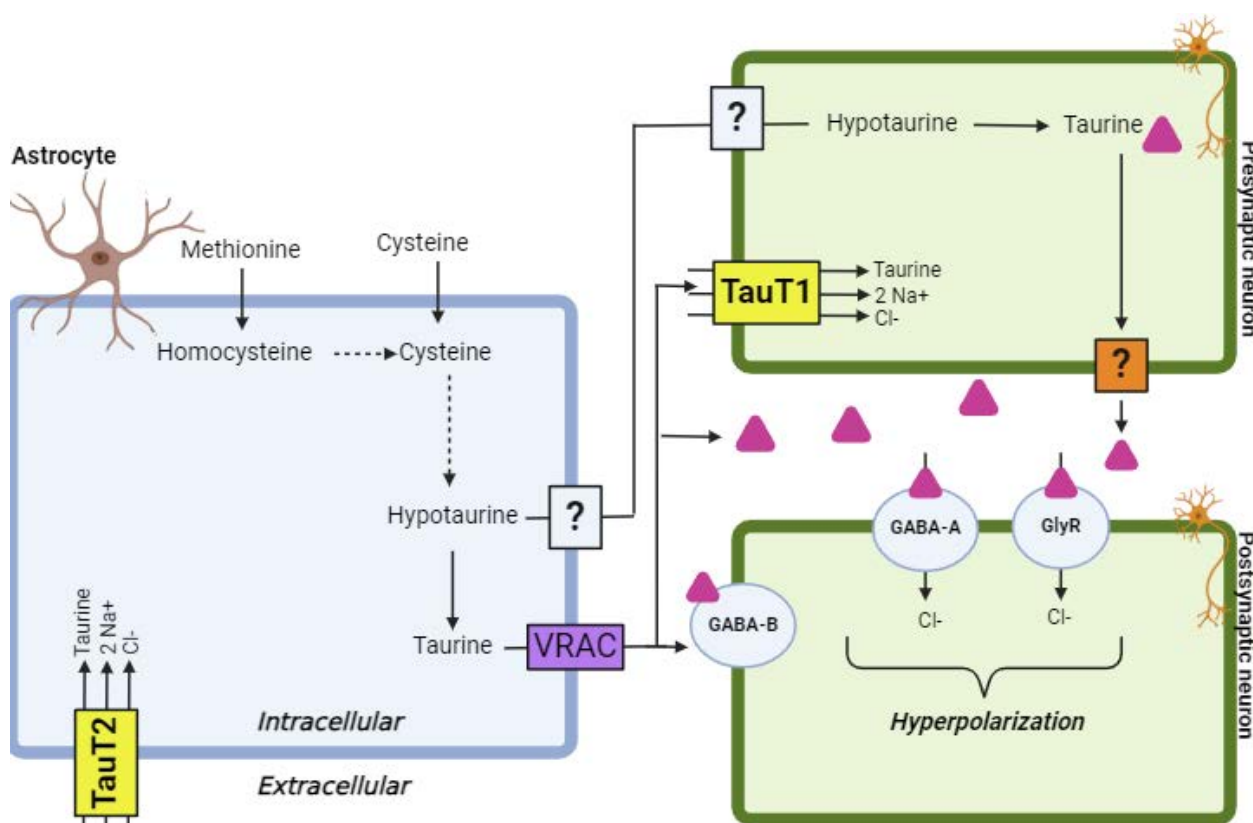


Figure 2. Biosynthesis of Taurine in mammals. Astrocytes produce taurine via three separate processes from methionine and cysteine from outside the cells^[2]. Hypotauroine or taurine is exported from the astrocyte to the extracellular space. However, the process by which hypotauroine moves from the astrocyte to the neuron is yet unknown. The transportation of taurine relies on the TauT1 and TauT2 transporters, exclusive to neurons and astrocytes, respectively. The process of releasing taurine from the pre-synaptic neuron has yet to be verified. Taurine is a complete activator for GABA_A, GABA_B, and glycine receptors in the post-synaptic neuron. This leads to an increase in chloride conductance, resulting in the hyperpolarization of the cell. There is ongoing debate over the existence of a taurine receptor on the post-synaptic membrane. Taurine is symbolized as a purple triangle. GlyR represents glycine receptor; TauT1 refers to taurine transporter type 1, TauT2 represents taurine transporter type 2, and an uncertain receptor/transporter is represented by a question mark (Created with [BioRender.com](https://www.biorender.com)).

Interestingly, a collaboration between astrocytes and neurons is proposed for the metabolism of taurine^[13]. Methionine, Cysteine, and Homocysteine form hypotauroine. Hypotauroine can be converted to taurine and released into extracellular space, or it can be released as hypotauroine and converted into taurine within a neuron. The TauT1 and TauT2 carriers carry out the transport of taurine. TauT1 is found in neurons and TauT2 in astrocytes^[2] (Figure 2). Taurine transporters regulate high levels of taurine in tissues with a low synthesis capacity. TauT is a Na⁺-Cl⁻-dependent

transporter^[14]. Neurons appear to be dependent on astrocytes for the synthesis of taurine as they do not have the enzyme cysteine sulfinic acid decarboxylase (CSAD), which converts cysteine sulphinic acid (CAD) to hypotaurine^[15].

Taurine is a full agonist for GABA_A, GABA_B, and glycine receptors^{[2],[17]-[19]}. Activation of these receptors by taurine leads to an increase in chloride conductance, resulting in the hyperpolarization of post-synaptic neurons^[2] (Figure 2). This means it can have an inhibitory effect, as gamma-aminobutyric acid (GABA) and glycine are inhibitory neurotransmitters. The concentration of taurine that can activate these receptors is 0.1 mM^[20]. An interesting study showed that taurine can protect hippocampal and cortical neurons from the effects of glutamate-induced excitotoxicity and β -amyloid peptide (A β) neurotoxicity, but the neuroprotection was no longer effective when introducing a GABA_A receptor antagonist^[21].

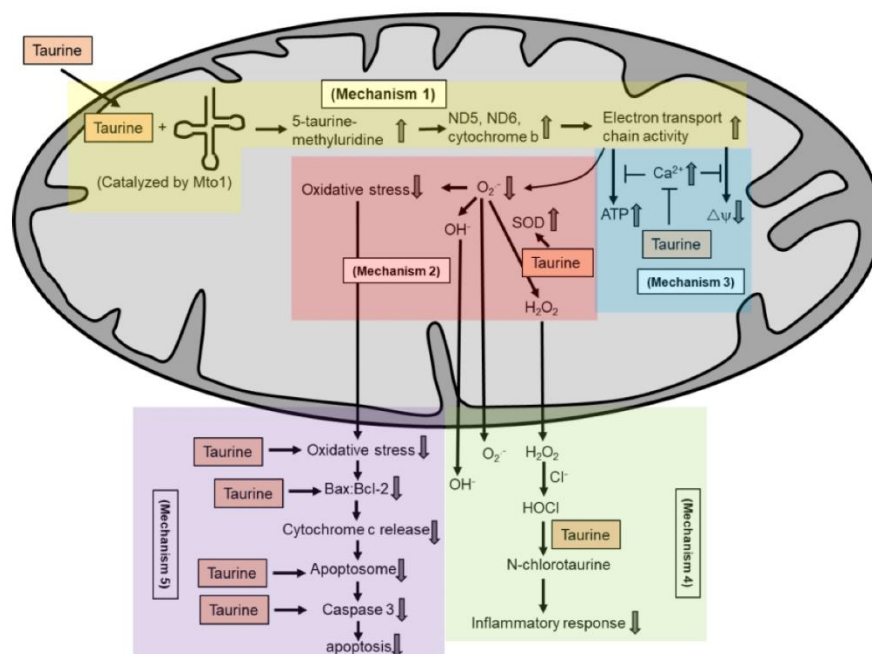


Figure 3. The important role of taurine in maintaining mitochondrial health: Potential mechanisms of action: (1) Taurine combines with uridine on mitochondrial tRNA to create a 5-taurinomethyluridine, essential for accurately producing mitochondrial proteins. This achieves the functionality of the respiratory chain complexes. (2) Taurine enhances the action of intracellular antioxidants, resulting in a reduction in superoxide production. (3) Taurine acts as a protective agent against excessive calcium accumulation and safeguards against a decrease in energy generation. (4) Taurine directly neutralizes hypochlorous acid (HOCl) by converting it into N-chlorotaurine, inhibiting a pro-inflammatory response. (5) Taurine prevents mitochondria-mediated apoptosis by inhibiting caspase activation or restoring the Bax/Bcl-2 ratio and blocking Bax translocation to the mitochondria. From Jong et al., 2021^[22], without changes (CC BY 4.0).

Taurine also has antioxidant activity. In their review, Jong et al., (2021)^[22] outline the mechanisms through which it exerts its antioxidant effects. These mechanisms include (Figure 3):

1. Taurine binding with uridine on mitochondrial tRNA to form 5-taurinomethyluridine. This modification is essential for the correct translation of mitochondrial mRNA into proteins, ensuring the proper functioning of the respiratory chain complexes.

2. Taurine enhances the activity of intracellular antioxidants, such as glutathione and catalase. This increased antioxidant capacity can lead to a reduction in the production of reactive oxygen species (ROS), including superoxide
3. Taurine acts as a protective agent against excessive calcium accumulation and safeguards against a decrease in energy generation.
4. Taurine directly neutralizes hypochlorous acid (HOCl), a potent oxidizing agent produced by immune cells. By converting HOCl into N-chlorotaurine, taurine can help to reduce oxidative stress and inflammation.
5. Taurine prevents mitochondria-mediated apoptosis by inhibiting caspase activation or by restoring the Bax/Bcl-2 ratio and blocking Bax translocation to the mitochondria. Taurine may therefore promote mitochondrial health through its antioxidant activity^[22].

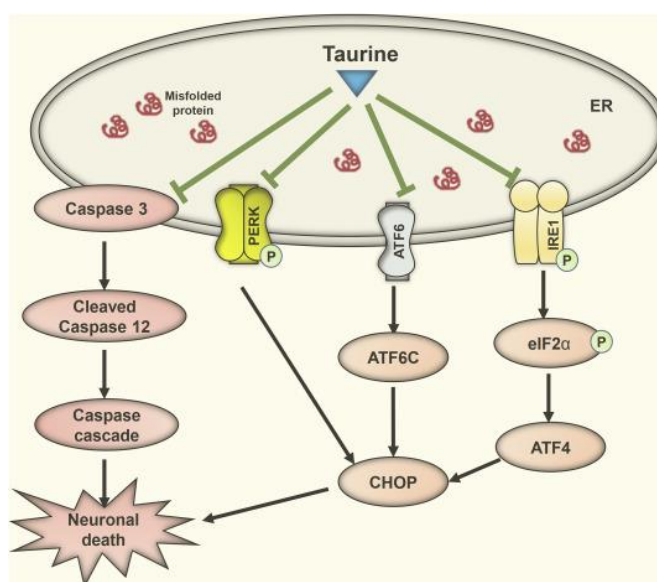


Figure 4. Taurine exhibits neuroprotective properties against endoplasmic reticulum (ER) stress mechanisms. ER stress triggers the activation of three specific kinds of ER membrane receptors, including PERK, ATF6, and IRE1. Phosphorylation of PERK leads to the conversion of eIF2α into phosphorylated eIF2α. This phosphorylated form of eIF2α then activates ATF4, which moves into the nucleus and initiates the transcription of CHOP. ATF6 undergoes conversion into its active form, known as cleaved ATF6, which is responsible for transcribing CHOP. Phosphorylation activates IRE1, which eventually enhances the production of CHOP. Caspase-12, situated on the external surface of the endoplasmic reticulum (ER) membrane, undergoes cleavage to transform into its active state, promoting the caspase cascade apoptosis process. Taurine prevents the activation of PERK, ATF6, IRE1, and caspase-12, exerting neuroprotective effects. From Jakaria et. al., 2019^[24], without changes (CC BY 4.0). ATF6: activating transcription factor 6, PERK: protein kinase RNA-like endoplasmic reticulum kinase, IRE1: inositol requiring enzyme-1, ATF6: activating transcription factor 6, CHOP: C/EBP homologous protein, eIF2: phospho-eukaryotic initiation factor 2.

Several studies have shown that taurine can effectively reduce inflammation in the brain. Prior research has demonstrated its efficacy in decreasing glial fibrillary acidic protein (GFAP) levels following experimentally generated traumatic brain damage (TBI). Furthermore, a one-week treatment with taurine effectively reduced the levels of 17 cytokines, including IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, tumor necrosis factor (TNF)-α, IFN-γ, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating

factor (GM-CSF), leptin, monocyte chemotactic protein-1 (MCP-1), and vascular endothelial growth factor (VEGF), in a rat model of traumatic brain injury (TBI). However, it only increased the level of macrophage inflammatory protein 1 alpha^{[23],[24]}. Furthermore, taurine reduced the neuroinflammation caused by microglia in a Parkinson's disease mouse model^[25]. These experiments also showed that taurine can prevent the activation of NADPH oxidase (NOX2) by disrupting the movement of the cytosolic subunit, p47phox, and the nuclear factor-kappa B (NF-κB) pathway. These two factors are crucial for starting and sustaining the M1 microglial inflammatory response^[25].

Taurine also significantly protects cells by reducing endoplasmic reticulum (ER) stress^[26]. The primary function of the endoplasmic reticulum (ER) is to facilitate the process of protein folding. Proteins that are not properly folded due to stress conditions are subsequently extracted from the protein folding process, transferred from the endoplasmic reticulum (ER) to the cytosol, and broken down in the ubiquitin-proteasome system through a process known as ER-associated degradation (ERAD). Accumulation of misfolded proteins over time will result in endoplasmic reticulum (ER) stress and trigger an adaptive reaction known as the unfolded protein response (UPR). The outcomes of UPR include the suppression of protein synthesis, the control of gene expression, and the determination of cell destiny, such as apoptosis, to fulfill the cell's requirements^[27]. Endogenous taurine functions as an anti-aging compound by promoting correct protein folding and lowering endoplasmic reticulum stress^[28]. [Figure 4](#) illustrates the protective effect of taurine against endoplasmic reticulum (ER) stress-induced neuronal injury.

1.2. Retina

The retina is the Central Nervous System (CNS) window to the world. It is an extremely complex tissue structure, consisting of many layers. Each layer has a defined type of cells. Its main function is to convert photons to electrical and chemical signals via neural pathways. The brain then processes these signals to create visual perception. The retina, located in the posterior region of the eye, functions as the innermost barrier among the other primary layers, including the vascular choroid and the fibrous sclera^[29]. The layers from the anterior to the posterior of the head are in the following order^[30] ([Figure 5](#)):

1. Inner limiting membrane
2. Nerve fiber layer (NFL)
3. Ganglion cell layer
4. Inner plexiform layer
5. Inner nuclear layer
6. Middle limiting membrane
7. Outer plexiform layer
8. Outer nuclear layer

9. External limiting membrane

10. The layer of rods and cones

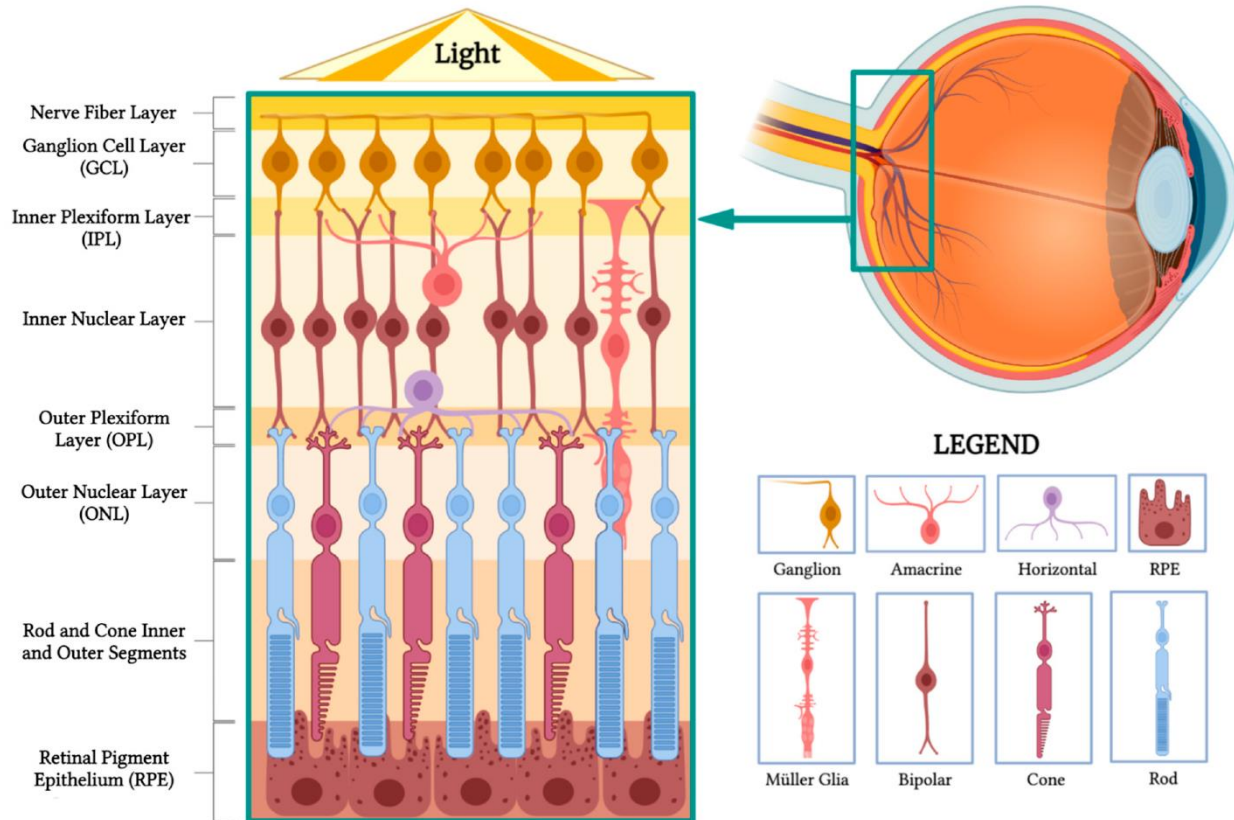


Figure 5. Anatomy of the retina. The picture emphasizes the several strata of the retina and its primary cellular components. **The nerve fiber layer** consists of the axons of retinal ganglion cells, which come together to create the optic nerve. **The ganglion cell layer** consists of the cell bodies of retinal ganglion cells, which are responsible for transmitting visual information to the brain. **The inner plexiform layer** is composed of the synaptic connections between bipolar and ganglion cells, which enable the processing and integration of signals. **The inner nuclear layer** contains the cell bodies of bipolar, horizontal, and amacrine cells, which are crucial for processing and conveying visual information. **The outer plexiform layer** is responsible for facilitating the early processing of visual information by including synapses between photoreceptors, bipolar cells, and horizontal cells. **The outer nuclear layer** consists of the cell bodies of rod and cone photoreceptors, which have the function of collecting and converting light into brain impulses. **The inner and outer segments of rods and cones** are components of photoreceptor cells. The inside segments of the cells include important biological components, such as mitochondria. On the other hand, the outside segments contain stacked discs that are rich in photopigments. These photopigments are crucial for absorbing light and starting the process of converting light into neural impulses, known as phototransduction cascade. **The retinal pigment epithelium (RPE)** is the outermost layer of the retina, positioned just below the photoreceptor cells. The Retinal Pigment Epithelium (RPE) plays a vital role in visual processing. The cells of this object absorb any light that is not in the intended direction, therefore reducing the scattering of light and improving the sharpness of vision. Additionally, they have a crucial function in the recycling of photopigments and the transportation of nutrients to the photoreceptors. In addition, they aid in the transportation of metabolic waste products from the photoreceptors to the choroidal blood supply, therefore contributing to the maintenance of the photoreceptor cells' health. Image from Wu et. al., 2023^[31], without changes (CC BY 4.0).

As stated, the retina consists of many layers that contain distinct types of cells, each with unique functions. These cells work together to convert incoming photons into electrical signals called action potentials. These signals are then processed by the brain's cortices to create a three-dimensional visual perception^[30]. The retina consists of six distinct neuronal cell types (Figure 5):

photoreceptors (rods and cones), retinal ganglion cells, bipolar cells, horizontal cells and amacrine cells.

1.2.1 Retinal Cell Types

Rods and Cones

Approximately 95% of the photoreceptors in the human retina are *rods*. Rods are specialized in detecting low-light levels and contribute to the creation of black and white vision, also known as scotopic vision. Rods are mostly located in the peripheral region of the retina, and their abundance gradually rises as one proceeds away from the central fovea. The central fovea, however, does not contain any rods. Rods exhibit distinct characteristics compared to their cone counterparts in terms of color perception. Rods converge into a single retinal ganglion cell (RGC) in greater numbers compared to cones, which maintain a 1:1 ratio. Rod cells use glutamate as their neurotransmitter to establish synaptic connections with second-order bipolar cells in the outer plexiform layer.

The human retina has roughly 6 to 7 million *cones*, which make up just 5% of all retinal photoreceptors. However, our *visual acuity* (VA), a metric that quantifies the eye's capacity to differentiate forms and discern the specific features of things at a specified distance^[32], depends on as few as 100,000 cones. Cones have lower overall sensitivity to photons compared to rods, but they excel at detecting light of one of three wavelengths, sometimes known as *hues*. Cone cells specialize in detecting specific wavelengths of light, with 64% sensitivity to red light, 32% to green light, and 2% to blue light. The *macula*, the center region of the retina that includes the fovea, predominantly concentrates these cells. The central fovea lacks both rods and synapses, only consisting of 100% cones that have a clear line of sight to the incoming light from the external environment. The *fovea* contains a ratio of 2:1 of red and green cone cells, respectively. In contrast, the cones in the peripheral region around the macula are responsible for detecting blue light. As mentioned above, cones in the macula have a synaptic link to retinal ganglion cells in a 1:1 ratio, while rods have a 3:1 ratio. This means that each cone contributes a larger quantity of visual input to the brain's interpretation of the total image. Cone cells release glutamate onto second-order bipolar cells situated in the outer plexiform layer^[30].

Retinal Ganglion Cells

Retinal ganglion cells (RGC) are the primary neurons responsible for transmitting information from the retina to the brain. There are over 20 distinct types of RGCs, and about 1 to 2% of all them have the ability to respond to light on their own. They serve as both photoreceptors and play a crucial role in transmitting non-image-forming information. This information is used in a variety of physiological processes, including the regulation of the circadian rhythm, modulation of melatonin release, and control of pupil size. Two types of intermediate neurons, amacrine cells and

bipolar cells, send signals to RGCs that can either stimulate or inhibit them. The suprachiasmatic nucleus, olivary pretectal nucleus, intergeniculate leaflet, ventral division of the lateral geniculate nucleus, and preoptic region are innervated by RGC axons, facilitating the coordination of circadian cycles and the pupillary light response^[30]. Retinal ganglion cells (RGCs) are specialized projection neurons that transmit an important amount of visual information from the retina to the brain. The full distance covered by retinal ganglion cells (RGCs) extends from the retina, beginning at synapses with bipolar and amacrine cells, to the brain, ending at synapses with neurons in the lateral geniculate nucleus^[33].

Amacrine Cells

Amacrine cells are a type of interneurons that release inhibitory neurotransmitters, either GABA or glycine. Due to their distinctive gap junction physiology, they can function as either inhibitory or excitatory. Amacrine cells exhibit significant heterogeneity and perform a wide range of roles and functions, making them the most versatile cells in the retina^[30]. What is the reason for the existence of several types of amacrine cells? There is a wide variety of amacrine cells due to the existence of several types of ganglion cells^[34]. Researchers have identified a total of 20 distinct ganglion cells, and more than 42 unique amacrine cell types. The diverse nature of amacrine cells allows for the formation of specialized functional microcircuits, which, in turn, enable the retina to detect certain hues and motions of light in specific directions. We categorize functional microcircuits based on the type of amacrine cells present, which include broad-field, medium-field, and narrow-field amacrine cells. The amacrine cell's information exchange varies across different fields, exhibiting various spectrums. Amacrine cells have both inhibitory and excitatory effects, even though they solely release inhibitory neurotransmitters. Amacrine cells can regulate signal transmission at different stages of the retinal pathway, either by inhibiting the release of neurotransmitters at the source (*pre-synaptic inhibition - action on bipolar cell terminals*), modifying how the signal is received (*post-synaptic inhibition - action on the dendrites of retinal ganglion cells*), or by forming electrical connections with other cells to influence neural activity directly. Certain amacrine cells exhibit a paracrine function; they release dopamine which acts as a neuromodulator influencing the activity of multiple retinal cells over a broader area^[30]. The Inner Nuclear Layer (INL) consists of the cell bodies of bipolar cells, horizontal cells, and amacrine cells. Amacrine cells play a critical role in regulating the flow of information between bipolar cells and RGCs, through their dendrites, which make synapses in the Inner Plexiform Layer (IPL) of the retina^[29].

Bipolar Cells

Bipolar cells are long projection neurons characterized by the 180° orientation of their axons to their dendrites. They receive visual inputs from photoreceptors and transmit them to the RGCs.

Thirteen distinct types of bipolar cells have been identified that are divided into rod bipolar cells or cone bipolar cells based on the source of their inputs. The cone bipolar cell and rod bipolar cell are classified based on their response to light, with some depolarizing (ON-bipolar cells) and others hyperpolarizing (OFF-bipolar cells). Cone bipolar cells exhibit either an ON or OFF type, while rod bipolar cells exclusively display the ON type. Bipolar cells form their synaptic connection with rods and cones in the retina's *outer plexiform layer (OPL)*, connecting the inner and outer cell layers. Bipolar cells establish unique connections with retinal ganglion cells (RGCs), amacrine cells, and horizontal cells. In the IPL, amacrine cells exert presynaptic inhibition on the terminals of bipolar cells. GABAergic horizontal cells provide inhibitory input to bipolar cells. Thus, bipolar cells get excitatory glutamatergic signals from rods and cones, as well as inhibitory GABAergic signals from horizontal cells. In return, bipolar cells transmit excitatory glutamatergic signals to RGCs and amacrine cells^[30].

Horizontal Cells

Horizontal cells regulate the transmission of information between bipolar cells and photoreceptors. Horizontal cells contribute to the adaptation of the eyes to varying levels of brightness. The retina has three differentiated kinds of horizontal cells, which have their cell bodies mostly situated in the inner nuclear layer (INL). Except for a small number of rodent species, such as rats and mice, which only have one kind of horizontal cell, most mammals have two types of horizontal cells^[35]. These cells are GABAergic and provide inhibitory inputs to bipolar cells, as well as inhibitory feedback to both rods and cones^[30]. Nevertheless, this is a topic that causes intense debate among academics. It is hypothesized that horizontal cells do not employ GABA for inhibition. Instead, they regulate the pH in the synaptic cleft to control bipolar cells and photoreceptors. Horizontal cells establish connections in the outer plexiform layer to transmit information about polarity, spectral sensitivity, speed, and spatial receptive field shape. Horizontal cells enhance signals from ON-OFF centers by delivering inhibitory GABAergic inputs to the neighboring bipolar cells that surround the ON-center or OFF-center bipolar cells in a circular pattern. Horizontal cells are crucial in enveloping bipolar cells, forming invaginating connections with dendrites of ON cone bipolar cells, and establishing basal connections with OFF cone bipolar cells^[30].

Müller Cells

Müller cells are the major type of glial cells in the human retina, topping astroglia and microglia. Müller cells were first described by Heinrich Müller. The human retina has 8–10 million Müller cells^[36]. The Müller cell is the only retinal glial cell that originates from the same cell lineage as retinal neurons, that is, a unique progenitor cell gives rise to both Müller cells and retinal neurons. Müller cells span the whole depth of the retina and establish connections with all types of neuronal cell bodies ([Figure 6](#)). The cell bodies of Müller cells are located in the inner nuclear layer (INL).

Like all glial cell types, Müller cells function as supportive cells for neurons while they enhance the formation of the internal blood-retinal barrier by stimulating the production of tight junctions and associated proteins in endothelial cells^[37].

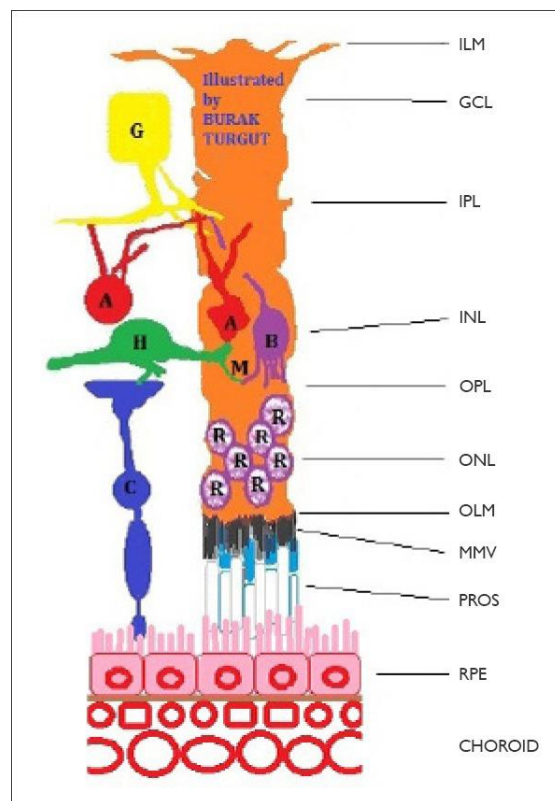


Figure 6. Graphic depicting the interconnection between a Müller cell and other neurons in the retina. Image from Kobat & Turgut, 2020^[37], without changes (CC BY 4.0). A: Amacrine cell, B: Bipolar cell, C: Cone cell, G: Ganglion cell, GCL: Ganglion cell layer, H: Horizontal cell, ILM: Inner limiting membrane, INL: Inner nuclear layer, IPL: Inner plexiform layer, M: Müller cell, MMV: Müller micro-villi, OLM: Outer limiting membrane, ONL: Outer nuclear layer, OPL: Outer plexiform layer, PROS: Photoreceptor outer segments, R: Rod cell, RPE: Retinal pigmented epithelium.

Müller cells provide neurons with trophic substances and remove metabolic waste (Figure 7). They have a crucial function in controlling the volume of the extracellular space in the retina, which is the area outside the cells where ions, nutrients, and waste products are found, maintaining a balance of ions and water, and preserving the integrity of the blood-retinal barrier. Müller cells help to clear neurotransmitters from the extracellular space around the synaptic clefts. These cells also play a role in the glutamate-glutamine cycle, which involves the absorption and processing of glutamate and GABA. This process combines neuronal stimulation with lactate production and other substances that nourish retinal neurons. Furthermore, Müller cells actively guard against oxidative and nitrosative stress^[38].

worsen disease progression by increasing blood vessel permeability and facilitating the entry of harmful substances. Chronic gliosis worsens the progression of the illness, increasing the permeability of blood vessels and facilitating the entry of harmful substances^[43]. Studies on animals lacking GFAP and vimentin indicate that reactive retinal glial cells play a significant role in contributing to retinal injury^[44].

Typically, Müller glial cells exhibit high levels of filamentous vimentin, while GFAP expression is relatively low. In a healthy retina, GFAP is predominantly found in astrocytes, the inner segments of Müller cells, and their terminal end-feet^[45]. Following retinal damage, Müller glial cells rapidly increase GFAP production. Previous studies have shown elevated GFAP protein levels in various models of glaucoma and retinal ischemia/reperfusion^{[46]-[49]}.

Astrocytes

Astrocytes are another type of glial cells. In 1893, Michael von Lenhossek introduced the name "astrocyte" to describe the star-shaped appearance of these cells, combining the Greek words "*ἄστρον*" for star and "*κύτος*" for cavity, cell^[50]. Astrocytes are a major cellular component of the central nervous system, comprising 20 to over 50% of the total number of cells^[51]. Astrocytes, along with other glial cells such as oligodendrocytes and microglia, contribute to the maintenance of the stability of central nervous system during several stages, including development, normal physiological functioning, and aging. In the retina, astrocytes are located in the ganglion cell layer ([Figure 8](#)). In contrast to Müller cells, which are found in the retinas of all vertebrates, astrocytes are only present in the mammalian retina^[52].

Astrocytes provide a range of essential functions that are crucial for the well-being of the CNS. These functions include maintenance of synapses and the blood-brain barrier (BBB) and blood-retinal barrier (BRB), providing neurotrophic support, and controlling blood flow^[53]. According to Holden and his colleagues (2023)^{[53][54]}, astrocytes have a far larger role in neurovascular interactions than what is generally recognized. One of their key functions is serving as templates for angiogenesis, which is critical for promoting blood vessel growth during brain development^{[54]-[56]}. In the retina, the development of blood vessels is regulated through the interactions among ganglion cells, astrocytes, and endothelial cells^[57].

During neural tissue damage, astrocytes undergo morphological, molecular, and functional changes known as reactive gliosis. These changes include increase of glial fibrillary acidic protein (GFAP) and vimentin^[58], production of inflammatory cytokines^[59], activation of JAK/STAT3 pathway^[60] etc. There are ongoing debates regarding the role of reactive astrocytes in central nervous system diseases, repair mechanisms, and aging. While GFAP is the primary marker used to identify reactive astrocytes, it is not the only one. Although an increase in GFAP mRNA and

protein expression is a prominent feature of many reactive astrocytes, it is not universally observed in all reactive astrocytes^[61].

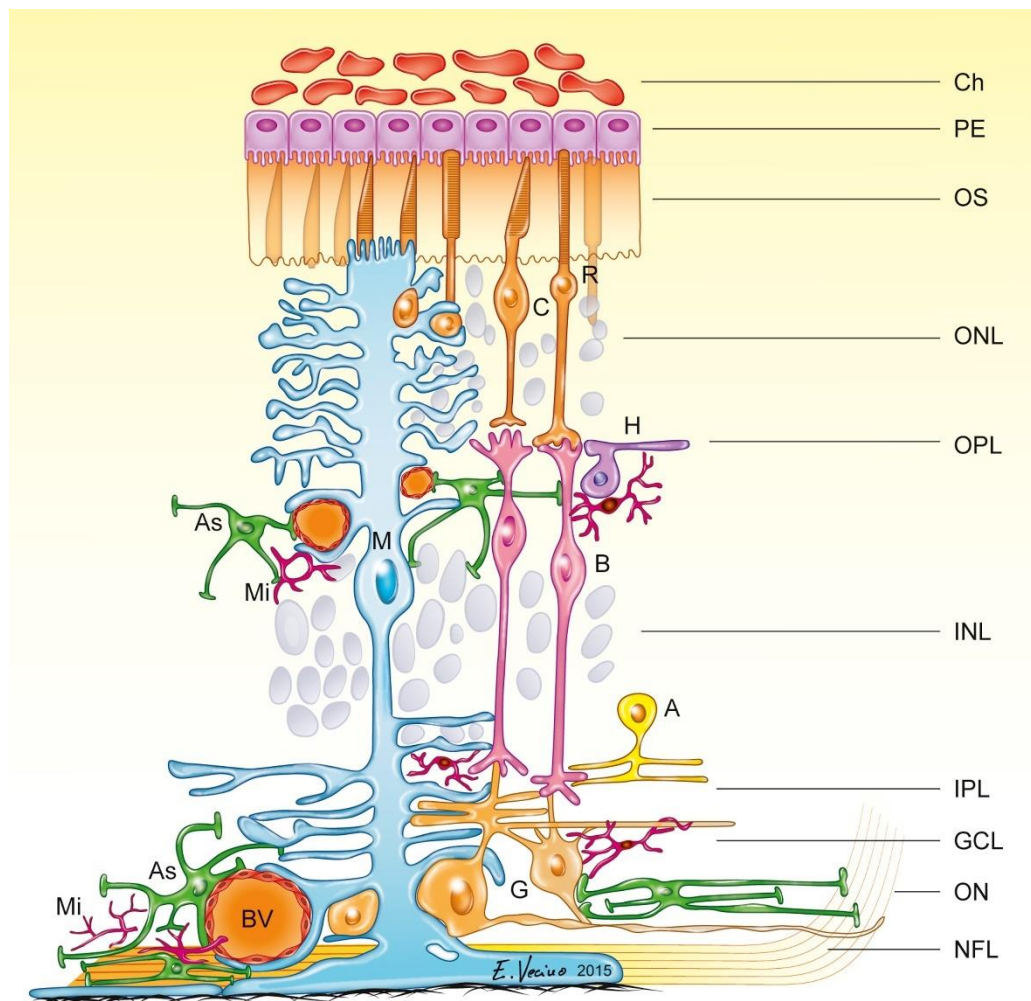


Figure 8. Structural elements of the retina. The retina's structural elements, such as glial cells and neurons, are illustrated. The many cell types are located within a typical, sizable mammalian retina. Observe the interactions between the cells and blood vessels (BV). The parts of the picture that have been labeled are: amacrine cells (A), astrocytes (AS) in green, bipolar cells (B), cones (C), ganglion cells (G), horizontal cells (H), Müller cells (M) in blue, microglia (Mi) in red, and rods (R). Look at how the different layers of the retina are arranged. From inside to outside, they are the optic nerve (ON), the nerve fiber layer (NFL), the ganglion cell layer (GCL), the inner plexiform layer (IPL), the inner nuclear layer (INL), the outer plexiform layer (OPL), the outer nuclear layer (ONL), the outer segment layer (OS), the pigment epithelium (PE), and the choroid (Ch). From Vecino et al., 2016^[50], without changes (CC BY 4.0).

Reactive astrogliosis has been studied in several situations such as normal aging^[62], Alzheimer's disease^{[63]-[65]}, Parkinson's Disease^[66], Autism^[67], and several eye diseases like Glaucoma^[68] and Diabetic Retinopathy^[69]. Thus, studying astrocytes, especially their morphological and molecular changes in pathology, is necessary. To use astrocytes as therapeutic targets, we need to focus on two objectives. It is necessary to clearly determine if reactive astrocytes and their associated signaling pathways play a substantial role in the development of various CNS diseases. The second hurdle involves the development of astrocyte therapeutics that are customized to address specific disease situations^[61].

Microglial cells

Microglial cells, the resident macrophages of the CNS, have long been recognized to be present in the retina^[70]. However, as aging occurs, the ability of microglia to maintain immune surveillance and support tissue regeneration declines^[71]. Microglia are thought to originate from monocytes^[72]. It is believed that microglia reach the retina by two possible routes: 1) by crossing the surface of the vitreal retina, or 2) by migrating from non-neural ciliary areas located in the peripheral areas of the retina^[73].

Microglia play a crucial role in various retinal processes, including neurogenesis^[74], synaptic function^[75], angiogenesis^{[76],[77]} and the regulation of inflammatory responses^{[78],[79]}. These cells continuously monitor the retinal environment and respond to neurodisorders. However, with aging, microglial function can become impaired, reducing their ability to contribute to tissue repair^[71]. In retinal degeneration, microglial inflammatory responses often become dysregulated^[80]. For instance, in a glaucoma model, inhibiting microglial activation was found to reduce retinal ganglion cell apoptosis caused by inflammatory cytokines^[81]. In diabetic retinopathy, microglia play a dual role. During the early stages of the disease, microglial activation is neuroprotective, releasing pro-inflammatory cytokines such as IL-1 β , IL-6, IL-8, and TNF- α . However, prolonged stress and excessive release of these molecules can worsen the condition by increasing inflammation^[82]. Thus, as mentioned earlier, microglia exhibit both protective and harmful effects in the brain and retina, depending on the stimuli associated with aging and retinal degeneration^[71].

1.2.2 Blood-Retinal Barrier

The Blood-Retinal Barrier (BRB) is a critical structure that regulates the exchange of substances between the bloodstream and the retinal tissue, protecting the retina from harmful agents while ensuring the high oxygen and metabolic substrate requirements of the tissue and a controlled environment for proper visual function. This barrier is divided into two components: the Inner Blood-Retinal Barrier (iBRB) and the Outer Blood-Retinal Barrier (oBRB; [Figure 9](#)), both characterized by the presence of tight junctions^[83]. The iBRB consists of endothelial cells lining the blood vessels in the inner retina. These vessels supply blood to the retina up to the outer plexiform layer, while the photoreceptor layer remains avascular. In contrast, the oBRB is formed by retinal pigment epithelial (RPE) cells and regulates the exchange of substances between the retina and the choroid^[83]. The development of the blood-retinal barrier is influenced by interactions between astrocytes and endothelial cells^[84]. Maintaining the BRB's integrity is essential, as its breakdown plays a key role in the development and progression of various retinal diseases^[83].

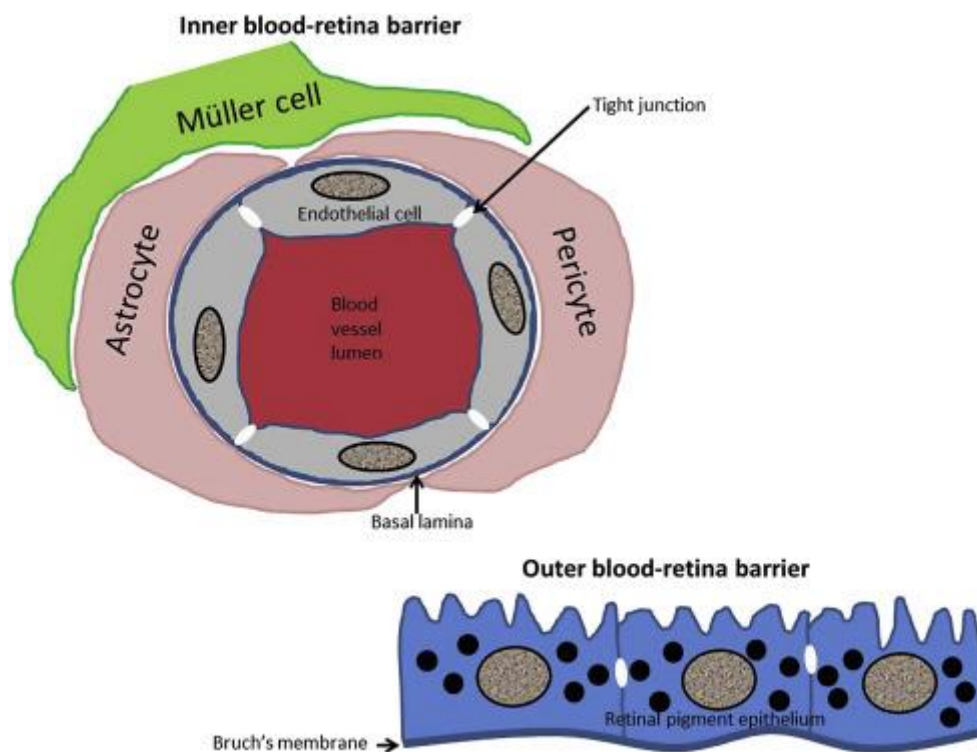


Figure 9. Structure of the inner and outer blood-retina barriers. Image from Nagymihály et al., 2021^[85]. (License Number: 5867561040543, License date: Sep 14, 2024)

1.2.3 Taurine in the Retina

Taurine is abundant in the retina where it accounts for almost half of the content of free amino acids^[86]. Several studies suggest that taurine deficiency leads to photoreceptor degeneration^{[87]-[91]}. The photoreceptor layer contains more than 60% of the taurine present in the retina^[92]. The neuroprotective property of taurine on the retina is further demonstrated by the finding that depletion of taurine leads to cellular damage in different layers of the retina, including retinal pigment epithelium cells, photoreceptors, and retinal ganglion cells^[93]. In conclusion, taurine protects the retina by several mechanisms, many of which are not fully understood.

Several studies are showing the value of taurine as a neuroprotective agent in the retina. Enhanced taurine feeding can directly improve the survival of retinal ganglion cells (RGCs) through intracellular pathways in a model of RGC degeneration^[92]. Further research suggests that administering taurine systematically can reduce the degeneration of photoreceptors in a Royal College of Surgeons (RCS) syndrome model of rat. These benefits are likely to be facilitated by many neuroprotective mechanisms. The rats who received treatment also exhibited a reduction in the quantity of microglial cells in the outer layers of the retina, a drop in the expression of glial fibrillary acidic protein (GFAP) in Müller cells, a decrease in oxidative stress in both the outer and inner nuclear layers, and an improvement in the preservation of synaptic connections^[91].

1.3. Ischemia/Reperfusion Injury

Ischemia is the reduced or blocked blood flow to a tissue, which deprives it of three essential components: oxygen, metabolic substrates, and the ability to remove waste products. If ischemia persists, it can result in tissue death. The process of restoring blood flow is known as reperfusion. However, reperfusion does not reverse the damage; in fact, it may worsen the condition. Ischemia/reperfusion injury (IRI) refers to the paradoxical phenomenon where cellular dysfunction and tissue death increase after blood flow is reestablished in previously ischemic tissues. This condition highlights the complexity of the damage caused by both ischemia and reperfusion^[94].

Several harmful processes lead to ischemia/reperfusion injuries, such as cell death (apoptosis, necrosis, and ferroptosis), oxidative stress, an inflammatory response, disruption of the blood-brain barrier (BBB), changes in the extracellular matrix (ECM), angiogenesis, cardiomyocyte hypertrophy, and fibrosis^[95]. Ischemia/reperfusion damage is the main cause of death in ischemic disorders, presenting an important challenge in developing successful treatment therapies. Despite the advancement of diverse treatment modalities such as antiplatelet and antithrombotic medicines, their efficacy in reducing ischemia/reperfusion damage remains constrained. Consequently, there is an urgent necessity for innovative therapeutic approaches to more effectively tackle ischemia disorders. Ischemia/reperfusion injury (IRI) implicates several signaling pathways. One of those is the Wnt pathway, as summarized by Zhang and his colleagues. In conjunction with other signaling pathways, the Wnt signaling pathway serves as a crucial regulator of the onset and advancement of I/R damage. Thus, addressing this signaling network is a promising treatment method for this pathology^[95].

1.3.1 Retinal Ischemia/Reperfusion Injury

The retina has the highest metabolic requirement of any tissue in the body and is particularly susceptible to ischemia damage^[96]. Degeneration of retinal ganglion cells (RGCs) is a major damage during retinal ischemia/reperfusion. This is a common feature of many eye diseases that can be very dangerous, like glaucoma and retinal vein occlusion^[97]. Following the termination of ischemia, reperfusion introduces oxygen and glucose, however, it also precipitates a significant release of free radicals and an increased inflammatory response. This then surpasses standard cellular antioxidant defense systems, ultimately resulting in cell death^[98]. For many years, research has focused on apoptosis as a type of cell death^{[97],[99],[100]}. However, recent research has revealed that various forms of cell death, including many forms of programmed necrosis, significantly

contribute to the pathophysiology of the disease^[101]. This is important because it presents the opportunity to test drugs and treat the disease.

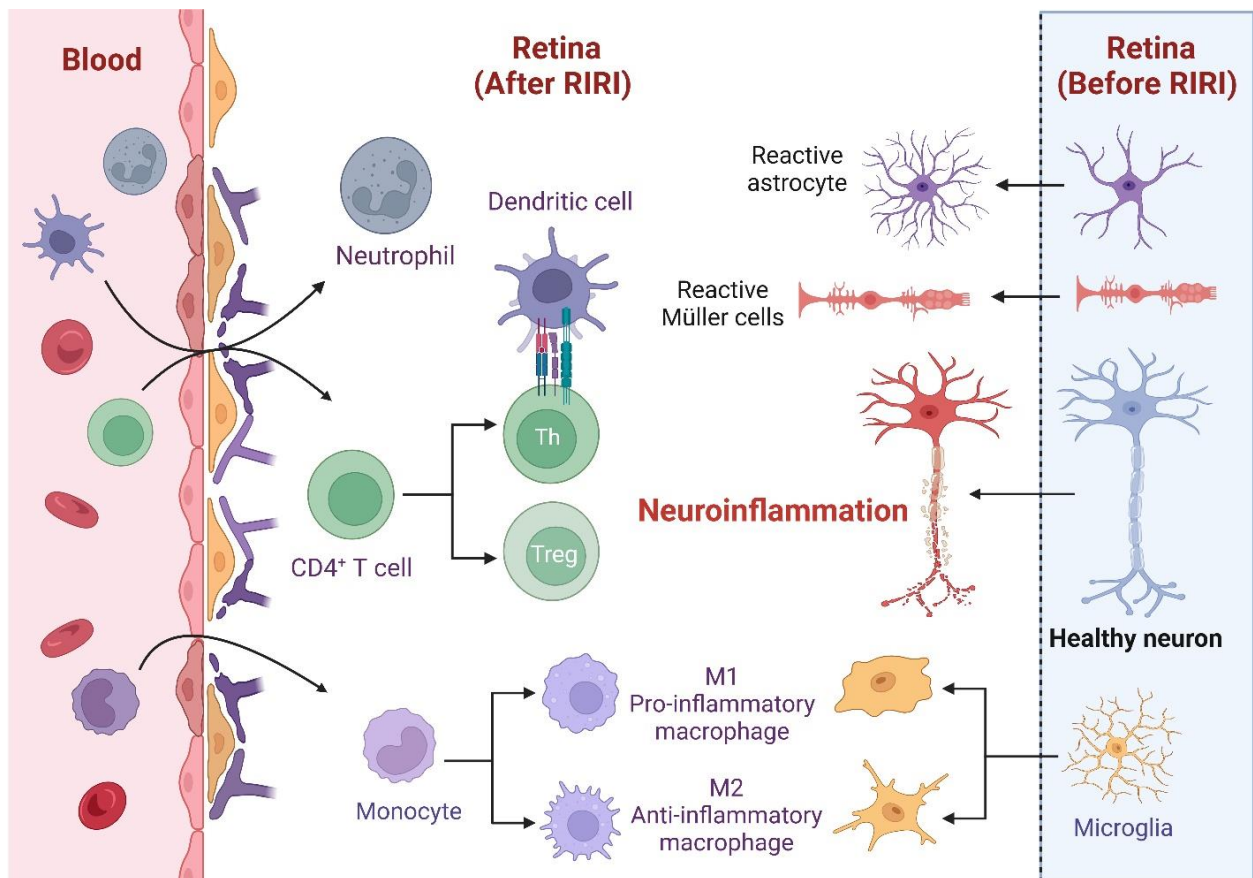


Figure 10. The illustration depicts the localization of immune cell populations within the retina. The illustration depicts immune cells, such as neutrophils, CD4⁺ cells, and monocytes, traversing the blood-retinal barrier to enter the retina. Furthermore, the illustration depicts resident immune cells in the retina, such as astrocytes, Müller cells, and microglia. Monocytes and microglia possess the capacity to develop into M1 and M2 phenotypes. From He et al., 2024^[96], without changes (CC BY 4.0).

The immune landscape associated with retinal ischemia/reperfusion injury is of special interest (Figure 10). The inflammatory response in RIRI is mostly mediated by peripheral immune cells and resident retinal immune cells, which are recruited to the injury site during the reperfusion phase^[96]. Microglia, the resident immune cells in the retina, monitor the retinal microenvironment and activate in response to disease or injury, changing morphology and producing pro-inflammatory cytokines. Activation of microglia may also be involved in neuronal death during RIRI^[102]. The overactivation of microglia can exacerbate inflammation^[103]. This mechanism leads to lymphocyte infiltration^[104]. The commonly used microglial marker Iba-1 fails to differentiate between microglia and entering monocytes. Upon activation, microglia/macrophages exhibit distinct morphological alterations and enhance the expression of activation markers. These cells produce pro-inflammatory cytokines (including TNF- α , IL-1 β , IL-6, and IFN- γ) and decrease the expression of anti-inflammatory proteins, such as TGF- β ^[96]. All the above illustrates how important proper regulation of microglia is for retinal health.

In harmful situations, like ischemia/reperfusion injury, astrocytes experience a phenomenon known as reactive gliosis, increasing the expression of intermediate filaments, namely glial fibrillary acidic protein (GFAP), vimentin, and nestin, resulting in enhanced rigidity. This defensive mechanism shields the retina from further damage. Nonetheless, sustained gliosis might increase damage, resulting in retinal degeneration^[96]. Research suggests a link between astrocytes and microglia. Activated neuroinflammatory microglia specifically produce reactive astrocytes^[105]. In summary, astrocytes in RIRI participate in inflammatory responses by releasing pro-inflammatory cytokines including TNF- α and in oxidative stress, which leads to neuronal injury and death^{[96],[106]}.

Retinal ischemia also precipitates Müller cell gliosis^[107]. The gliosis of Müller cells has both cytoprotective and cytotoxic effects on retinal neurons^[108]. Although microglia have been extensively studied in the context of retinal inflammation, increasing data indicates that Müller glia are essential in regulating this inflammation^[109]. Müller cells have been demonstrated to be a significant source of retinal cytokines^[110]. Furthermore, active microglia have been demonstrated to directly impact Müller cells, and the responses of Müller cells may enhance early inflammatory reactions^[111]. A recent study revealed a primarily pro-inflammatory type of Müller cells. The cells released several pro-inflammatory cytokines, complement components, and increased proteins linked to antigen processing and presentation, indicating a role as atypical antigen-presenting cells during retinal inflammation^[109].

1.3.2 Taurine and Retinal Ischemia/Reperfusion Injury

Taurine has not been previously evaluated in retinal ischemia/reperfusion injury (RIRI); nevertheless, substantial data supports its protective mechanisms against ischemia/reperfusion disease in other organs, including the brain. It demonstrates antioxidative capabilities, stabilizes membranes, acts as an osmoregulator, modifies ionic transport, decreases pro-inflammatory levels, and regulates intracellular calcium concentration, all of which contribute to its neuroprotective impact against ischemic stroke^[112].

A 90% decrease in stroke incidence was noted in a genetic rat model of stroke (SHRSP: Stroke-prone spontaneously hypertensive rat) that was provided with a taurine-enriched fish diet^[113]. Furthermore, in a rat model of experimental cerebral ischemia induced by 2 hours of middle cerebral artery occlusion (MCAO), taurine was seen to produce a neuroprotective effect by upregulating calpastatin and downregulating calpain and caspase-3. The neuroprotection exhibited a dose-dependent effect^[114]. Additionally, an *in vitro* study showed that taurine decreases the production of caspase-8 and caspase-9 produced by ischemia in the hypothalamus nuclei of

mice^[115]. Excitotoxicity is regarded as the primary cause of cellular death in ischemic stroke^[116]. Taurine has protective properties against glutamate-induced neuronal damage because it reduces the glutamate-induced increase in intracellular free calcium levels^[117]. Taurine also protects against glutamate excitotoxicity through the activation of GABA_A and strychnine-sensitive glycine receptors^[118].

We conclude that multiple research studies demonstrate the neuroprotective effects of taurine in ischemia/reperfusion injury.

1.4. *In silico* and Network Pharmacology

1.4.1 *In silico* Pharmacology

The development of "-omics" sciences establishes systems biology approaches as innovative paradigms in pharmaceutical research, enabling the discovery of new targets and therapies for complex multifactorial disorders. *In silico* Pharmacology involves the use of computer simulations, algorithms, and models to predict how drugs interact with biological systems. It involves techniques like molecular docking, virtual screening, and pharmacokinetic modeling to predict drug behavior, efficacy, and safety. This approach is primarily focused on predicting individual drug interactions with specific molecular targets, such as receptors or enzymes, before conducting laboratory or clinical experiments. It accelerates drug development by reducing the need for early-phase experimental testing. *In silico* Pharmacology is commonly used for drug discovery, toxicity prediction, and optimization of drug candidates^[119].

1.4.1 Network Pharmacology

Network Pharmacology on the other hand, is an approach that focuses on understanding how drugs affect complex biological networks^{[120],[121]}. It integrates systems biology, bioinformatics, and pharmacology to study the interactions between multiple targets, pathways, and diseases, especially for multifactorial diseases. Rather than looking at a single drug-target interaction, network pharmacology considers how drugs influence entire networks of proteins, genes, and pathways. It emphasizes a multitargeted approach, recognizing that many diseases like cancer, ischemia, or neurodegenerative disorders involve multiple biological pathways^[122]. Network Pharmacology is useful for researching complex diseases like retinal ischemia/reperfusion injury, where understanding the interaction of various biological pathways is crucial. The primary benefit of this approach is the simultaneous analysis of huge numbers of genes, proteins, and molecules

in a single experiment. This enables the measurement of established signaling pathways and biological networks fully, as well as the identification of new pathways and processes previously unreported. However, the large amount of produced data could complicate the differentiation between the real causes and extra background noise^[123].

Several studies using Network Pharmacology methodology have emerged in recent years, addressing various disorders like cancer, diabetic retinopathy, cervical spondylotic myelopathy etc.^{[124]-[126]}. As far as ischemia/reperfusion injury is concerned, network pharmacology has been proven useful in the study of the effects of drugs and natural produces on the ischemia/reperfusion injury of various tissues ([PubMed Search](#)). Employing Network Pharmacology on multifactorial pathologies in general and retinal ischemia/reperfusion injury in particular can help in (1) identifying multiple drug targets within the disease network, (2) understanding drug effects on multiple pathways, rather than just one and (3) predicting potential side effects or *off-target* effects by observing the drug's influence on the entire network.

1.5 Aim

The aim of this thesis was to create a robust framework for studying the effects of taurine on retinal ischemia/reperfusion injury, and to develop a versatile *in silico* model widely applicable in drug-target evaluation for complex multifactorial diseases.

Specifically, our goals were the:

1. **Development of a *state-of-the-art in silico* model for evaluating pharmacological targets in the treatment of multifactorial diseases** based on correlating molecular mechanisms involved in such diseases with potential drug targets, using retinal ischemia/reperfusion injury as the reference pathology and taurine as the reference bioactive compound.
2. **Creation of a drug-target-disease network** for the identification of key potential targets of taurine for the treatment of retinal ischemia/reperfusion injury.
3. **Validation of *in silico* identified targets** through molecular docking studies.
4. **Assessment of taurine's effects on astrocyte activation** at two different time points after retinal ischemia/reperfusion injury using an *in vivo* rat model.
5. **Assessment of taurine's effects on its *in silico* identified targets** at two different time points after retinal ischemia/reperfusion injury using the *in vivo* rat model.

2. METHODOLOGY

2.1 Network Pharmacology

As mentioned earlier, Network Pharmacology can provide a systematic and comprehensive perspective on the potential mechanisms of action of taurine in treating retinal ischemia/reperfusion injury, allowing the discovery of novel therapeutic targets beyond the traditional pathways. This approach can also guide further experimental validation [see below: *molecular docking* (2.2) and *in vivo studies* (2.3)] for effective drug development^{[120],[121]}. The approach followed was similar to that employed by He and colleagues (2023)^[127] and involved of the following steps.

2.1.1 Identification of Possible Taurine Targets

The possible targets of taurine were obtained from the various databases and webtools (Table 1):

Table 1: Sources of possible taurine-related targets *Databases were accessed between January 15 and March 19, 2024*

Resources	Description
Databases	
<u>DrugBank</u> ^[128]	A database which focuses on approved drugs, providing detailed information on their pharmacological properties, mechanisms of action, clinical trials, and adverse effects. While the primarily focus of DrugBank is approved drugs, it also includes some naturally occurring compounds like taurine.
<u>PubChem</u> ^[129]	A database that integrates information from diverse sources, including scientific publications, experimental data, and computational predictions.
<u>ChEMBL</u> ^[130]	A large-scale bioinformatics database that focuses on drug-like small molecules. It includes both approved and experimental drugs, as well as bioactive molecules not yet developed into drugs. It provides information on their bioactivity data, chemical structures, and target information.
<u>ChemSpider</u> ^[131]	A chemical database that provides detailed information on the chemical structures, properties, and bioactivity of compounds.
<u>RCSB</u> ^[132]	It contains structural data on protein-ligand complexes, that help to understand how a compound might bind to specific proteins and to predict potential biological targets.
<u>CHEBI</u> ^[133]	It provides cross-references to other databases and resources that allows access to broader information regarding the biological roles, biochemical pathways, and pharmacological effects of various chemical entities.
<u>Stitch</u> ^[134]	It is designed to explore the interactions between small molecules and proteins. It integrates information from various sources, such as experimental data, computational predictions, and text mining, to map out networks of chemical-protein interactions.
Web Services	
<u>GalaxyWeb</u> ^[135]	GalaxyWeb includes tools for various bioinformatics applications. GalaxySagittarius compares the chemical structure of a compound to other

([GalaxySagittarius](#) & [GalaxySagittarius-AF](#))

known drug-like compounds with established protein targets. Based on these similarities, it predicts potential protein targets for the compound. GalaxySagittarius-AF utilizes AI-powered predicted protein structures (AlphaFold models) to analyze how a compound might interact with different proteins at the atomic level. This approach offers more theoretical predictions based on direct analysis of potential binding interactions but relies on the accuracy of the AlphaFold models for the proteins of interest.

[SwissTargetPrediction](#)^[136]

This tool predicts potential targets of bioactive molecules. It doesn't rely on existing data but uses computational methods to suggest possibilities based on molecular similarities.

To standardize protein identifiers found in the different databases and webtools, [g:Profiler](#)^[137] and [SynGO](#)^[138] were used to convert all identifiers -regardless of the source species- to the respective human gene aliases. Double entries were excluded using the Excel 365 UNIQUE function.

2.1.2 Identification of Possible Ischemia/Reperfusion Injury-Associated Targets

There is a wealth of data in various databanks regarding the effects of ischemia and/or reperfusion injury in the brain and other tissues; however, information on retinal ischemia/reperfusion injury is relatively sparse. Using this wealth of information, we initially identified potential targets associated with ischemia/reperfusion (I/R) injury. These targets were sourced from the databases shown in [Table 2](#). The search terms varied based on the disease entities found in the different databases. Search terms used were: '[Brain Ischemia](#)', '[Reperfusion Injury](#)', and '[Ischemia AND Reperfusion AND Brain](#)', '[Ischemia](#)', '[Reperfusion](#)' and '[Ischemia/reperfusion](#)'.

Table 2: Sources of possible taurine-related targets *Databases were accessed between January 15 and March 19, 2024.*

Databases	Description
DisGeNET ^[139]	A database that focuses on the relationship between genes and diseases. It integrates data from various sources, including scientific literature, databases, and clinical studies, to provide a rich resource for researchers.
Genecards ^[140]	It provides detailed information about human genes including insights into diseases associated with each gene.
Therapeutic Target Database ^[141]	A specialized resource that focuses on therapeutic targets related to human diseases

To standardize protein identifiers found in the different databases and webtools, [g:Profiler](#)^[137] and [SynGO](#)^[138] were used to convert all identifiers -regardless of the source species- to the respective human gene aliases. Double entries were excluded using the Excel 365 UNIQUE function.

2.1.3 Identification and Functional Classification of the Common Taurine and Ischemia/Reperfusion Injury Targets

The [Draw Venn Diagram](#) webtool was used to identify potential therapeutic targets for taurine in ischemia/reperfusion injury. Common targets were found at the intersection between the taurine targets and disease targets. The functional classification of the possible taurine therapeutic targets was conducted using the *SIGnaling Network Open Resource 3.0* ([SIGNOR 3.0](#); score > 0.70) webtool^[144]. SIGNOR 3.0 focuses on signaling pathways and molecular interactions, allowing for a detailed understanding of specific biological processes influenced by taurine.

2.1.4 Identification of Potential Therapeutic Targets of Taurine at Different Time Points of Rat Retinal Ischemia/Reperfusion Injury

A [rat retinal ischemia/reperfusion injury time-dependent gene profiling](#)^[142] was recovered from the [EMBL-EBI Gene Expression Atlas](#) and a Venn diagram was plotted to identify the potential therapeutic targets of taurine at different time points (1 and 7 days after retinal I/R injury).

2.1.5 Construction and Analysis of Protein-Protein Interaction Networks

The possible therapeutic targets for taurine against I/R injury or the different rat retinal I/R time points were uploaded to the [STRING](#) webtool to construct Protein-Protein Interaction (PPI) Networks. The PPI networks with a confidence score greater than 0.70 were exported from STRING and imported into [Cytoscape](#) software^[143] (version 3.10.2) for analysis. The [cytoHubba](#) plugin was utilized to identify significant nodes (*core targets*) within these biological networks. Four ranking methods/topological algorithms were employed:

1. Degree: this method measures the number of connections (edges) a node has within a network. Nodes with a higher degree are considered more central or influential because they interact with more other nodes. It's a simple and straightforward metric for identifying key players in a network.
2. Maximum Neighborhood Component (MNC): MNC evaluates the size of the largest connected subnetwork that can be formed by including a specific node and its neighbors. It identifies nodes that are not only well-connected but also link to other important nodes, making them crucial for maintaining network integrity.
3. Maximal Clique Centrality (MCC): This method focuses on identifying nodes that are part of the largest cliques (*fully connected subgroups*) within the network. Nodes that appear in

many cliques are considered significant because they serve as bridges among groups of interacting partners, highlighting their role in facilitating communication within the network.

4. **Closeness**: Closeness measures how close a node is to all other nodes in the network. It calculates the average shortest path length from a node to all other nodes, indicating how quickly information or signals can spread from that node. Nodes with high closeness can rapidly influence or be influenced by other nodes, making them key players in network dynamics.

Specifically, the top 15 taurine targets identified by each ranking method were then analyzed using the [Draw Venn Diagram](#) webtool to find unique molecules (core targets). Core targets were subsequently subjected to molecular docking studies to confirm their possible direct interactions with taurine (*see: 2.2 Molecular Docking*). In future studies, the protein levels of core targets will be measured in tissue samples obtained from the *in vivo* retinal ischemia/reperfusion experiments performed (*see: 2.3*) to confirm taurine's effects on them at the two time points of retinal ischemia/reperfusion injury studied (*see: 2.1.4*). These molecules were also introduced in the *SIGNALING Network Open Resource 3.0* ([SIGNOR 3.0](#); score > 0.90) webtool ^[144] to identify the potential pathways they are involved at.

2.1.6 Elucidation of Taurine's Mechanisms of Action in Ischemia/Reperfusion Injury Using KEGG and GO Enrichment Analyses

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses help interpret large datasets and uncover potential mechanisms of action related to drug effects. Enrichment Analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) helps identify which biochemical and/or signaling pathways are significantly affected by taurine in the context of ischemia/reperfusion injury. By mapping genes or proteins influenced by taurine to known pathways, KEGG enrichment can reveal how taurine modulates processes such as oxidative stress, inflammation, or cell death. On the other hand, Gene Ontology (GO) analysis categorizes the genes or proteins influenced by taurine into three GO categories—biological processes, cellular components, and molecular functions. For ischemia/reperfusion injury, GO enrichment can reveal how taurine impacts specific processes (*e.g., apoptosis, neuroprotection*), cellular structures (*e.g., retinal layers*), and functions (*e.g., ion transport or antioxidant activity*).

The [Metascape](#) platform was used to perform both KEGG and GO enrichment analyses^[145]. The taurine and ischemia/reperfusion injury overlapping targets were uploaded to the platform, with the following parameters:

1. Min Overlap = 3 (*the minimum number of overlapping genes required between the target list and a biological category e.g., a molecular function or pathway to consider it for analysis*),
2. P-Value Cutoff = 0.01 (*any result with a p-value<0.01 is considered statistically significant*), and
3. Min Enrichment = 1.5 (*only pathways or functions where the observed number of overlapping genes is at least 1.5 times greater than expected by chance is considered enriched*).

After the enrichment analyses, the clustering network was exported and categorized according to the P-value.

2.2 Molecular Docking

Molecular docking is a computational technique used to predict the interaction between a small molecule and a target protein, and to assess the binding affinity of compounds like taurine to their target proteins. For this study, taurine's structure was retrieved from the PubChem database and converted to PDB format using [Open Babel](#) software^[146]. Docking simulations were performed using [AutoDock Vina 1.1.2](#)^[147], integrated into the [UCSF Chimera 1.17.1](#) software^[148]. The resulting interactions were visualized with UCSF Chimera.

Docking methods need each atom to be charged. "Dock Prep," a molecular docking feature of UCSF Chimera, prepares the ligand and target protein for docking simulations. It includes adding hydrogens and designating charges, which may assist in positioning of the ligand inside the protein's binding site. The steps followed to prepare the protein and ligand were as follows:

1. File > Fetch by ID > *Write the ID of protein* > *Click Fetch*
or Tools > Structure Editing > Build Structure > PubChem CID > *Write the CID of the ligand of interest (1123 for taurine)* > *Click Apply and Close*
2. Tools > Surface/Binding Analysis > Dock Prep > *Check all options except "Delete non-complexed ions" and "Write Mol2 file"* > *Confirm with OK* > *At the next window check "Consider each model in isolation from all others", Choose "Also consider H-bonds (slower)" and "Residue-name-based)"* > *Confirm with OK* > *At the next window check*

“Standard residues: AMBER ff14SB”, and “Other residues: Gasteiger” > Confirm with OK

3. *Save > Save PDB... > Choose the location > Click “Save”*

The key steps followed to visualize and assess taurine-target interactions are:

1. *File > Open > Load the Target Protein*
2. *Presets > Publication 1*
3. *File > Open > Load Ligand (Taurine)*
4. *Favorite > Sequence > Info > Uniprot Annotations > Enter Uniprot ID > Confirm with OK*
5. *Move the ligand outside the target area*
6. *Tools > Surface/Binding Analysis > AutoDock Vina > Make the correct matching for receptor and ligand, Click resize search volume option, Choose button 3, and with the right click create a square for docking. At the executable location select the location of the AutodockVina.*
7. *Tools > Structure Analysis > Find Clashes/Contacts > Confirm with OK*
8. *Actions > Label > Residue > Custom > Name + Number > Confirm with OK*
9. *Actions > Color > Select Options > Residue Labels: Black > Close*
10. *Select > Chain A (Target Protein)*
11. *Tools > Depiction > Rainbow*
12. *Actions > Surface > Show*
13. *Actions > Surface Transparency > Set to 80% > Confirm with OK*

2.2.1 Clash/Contact Analysis

Clash/Contact analysis is a critical step in molecular modeling that identifies steric clashes and favorable interactions between atoms in a molecular structure. This analysis helps in understanding how well a ligand, like taurine, might fit into a target protein's binding site. Clashes suggest unfavorable interactions that might destabilize the binding pose. In a docking study, reducing these clashes through ligand repositioning or protein flexibility is crucial for achieving a stable complex. On the other hand, contacts represent favorable interactions. For example, if a taurine molecule forms hydrogen bonds or hydrophobic interactions with the target protein, these contacts stabilize the binding pose and contribute to the binding affinity.

For the Clash/Contact analysis, we defined specific **threshold settings** to identify steric clashes and favorable contacts between atoms. Threshold settings choices influence how interactions are

detected and interpreted. The threshold settings to identify steric clashes and favorable contacts between atoms were as follows ([Figure 11](#)):

Clash/Contact Parameters

1. **Van der Waals (VDW) overlap ≥ 0.1 Å:** This setting determines how much closer two atoms can approach compared to the sum of their Van der Waals radii before being flagged as a clash. A threshold of 0.1 Å is relatively sensitive, capable of detecting minor steric conflicts, though it may also highlight marginal cases where slight adjustments in atom positions could eliminate the clash.
2. **Subtract ‘-0.2’ from overlap for potential H-bonding pairs:** Hydrogen bonds are generally shorter than standard Van der Waals interactions, so applying this subtraction allows atoms to be closer when a hydrogen bond is likely. Setting this value to -0.2 Å accounts for the typically shorter distances observed in hydrogen bonds.
3. **Default clash/contact criteria:**
 - i. **Ignore contacts of pairs 5 or more bonds apart:** This setting excludes interactions between atoms that are part of the same molecule or chain but separated by five or more bonds. This is a common practice to eliminate trivial contacts within a single residue or molecule, ensuring that the analysis focuses on significant inter-molecular or inter-residue interactions rather than internal atomic contacts that do not influence binding.
 - ii. **Include intra-residue contacts:** This option considers interactions within a single residue, such as those between atoms in the same amino acid. Including these contacts can offer insights into local conformational strain, which can be valuable for detailed structural analysis. However, they generally have limited significance when it comes to ligand binding.
 - iii. **Include inter-residue contacts:** This option considers interactions between atoms in different residues, regardless of whether they belong to the same protein or to different molecules (such as ligand-protein interactions).

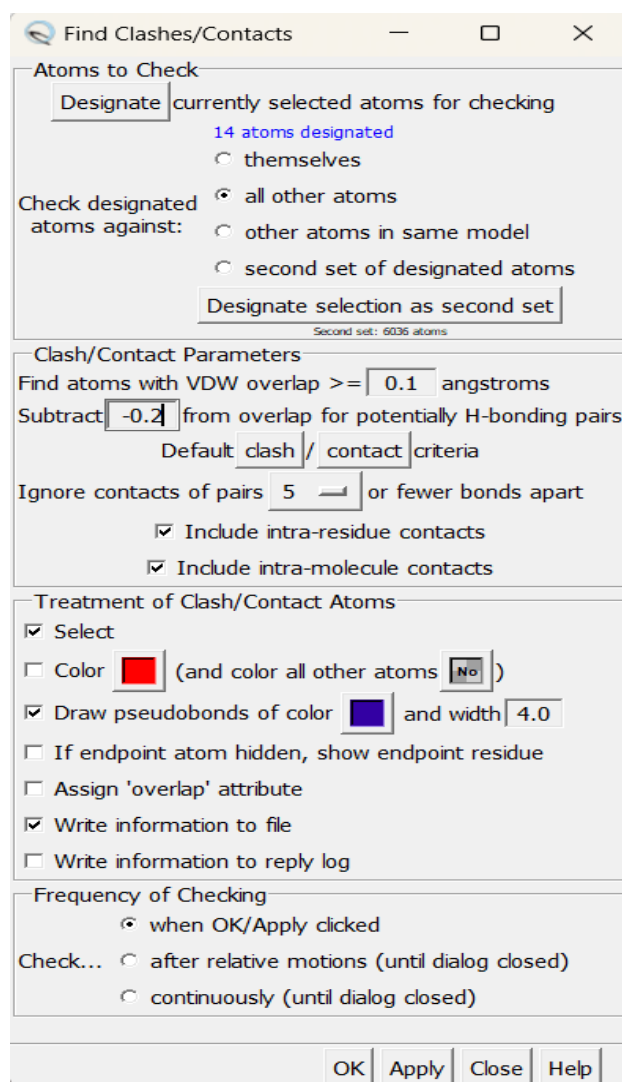


Figure 11: Clash/Contact analysis threshold settings applied for the identification of taurine-target protein interactions during molecular docking. The "Find Clashes/Contacts" tool in UCSF Chimera helps detect interactions, allowing to refine the docking results and visualize how a ligand and a protein interact in both favorable (contacts) and unfavorable (clashes) ways. Clashes occur when atoms or molecules are too close to each other, resulting in steric hindrance. In the context of molecular docking, clashes indicate unfavorable or physically impossible interactions because the atoms are overlapping or violating Van der Waals radii. These need to be minimized for a realistic and stable docking pose. Contacts refer to atoms or residues that are in close proximity and within a range where they can interact favorably, such as through hydrogen bonding, hydrophobic interactions, or other non-covalent interactions. These interactions are crucial for stabilizing the binding of the ligand to the target protein.

Treatment of Clash/Contact Atoms

1. **Select, draw pseudobonds of color 'blue' and width '4.0':** This option visualizes interactions by creating blue pseudobonds between clashing or contacting atoms. The blue coloring and 4.0 pixel thickness serve as a clear visual indicator, facilitating easy identification of these interactions in the 3D viewer of UCSF Chimera.
2. **Write information to file:** This feature generates a detailed report of all detected clashes and contacts, including atom IDs, residue names, and distances, and saves it to an output file. This information is valuable for further analysis, providing precise data on the

locations of clashes and contacts, which can help in refining the molecular model as needed.

Interpretation of spatial relationships

The information file generated by the "Find Clashes/Contacts" tool ([Figure 11](#)) of UCSF Chimera contains detailed data about the spatial relationships between atoms, including both favorable and unfavorable interactions:

- 1. List of Clashes:** This section shows *clash pairs* that is, pairs of atoms (*from the ligand and the target protein*) that are too close to each other, resulting in steric clashes. The *distance* between the two clashing atoms is reported, and it is typically smaller than the sum of their Van der Waals radii, indicating an unfavorable interaction. Sometimes, the file may also include the *severity* of the clash, which indicates how much the atoms overlap or violate spatial constraints. Severe clashes indicate significant steric hindrance.
- 2. List of Contacts** This section shows *contact pairs* that is, pairs of atoms (one from the ligand and one from the target protein) that are close enough to interact but not too close to cause a clash. The *distance* between atoms involved in the contact is within the favorable range for non-covalent interactions (e.g., hydrogen bonds, van der Waals interactions, hydrophobic interactions). Sometimes, specific *types of interactions* (e.g. *hydrogen bonds, ionic interactions*) are indicated based on the distances or atom types.
- 3. Residue Information** The tool may list which *residues* from the protein are involved in clashes or contacts with the ligand. Residue names, numbers, and chain IDs are provided, indicating which specific parts of the protein are interacting with the ligand. This helps identify key residues in the protein that are important for ligand binding. For example, taurine contact with a key residue in the binding pocket could suggest an important interaction that contributes to the biological activity of taurine.
- 4. Threshold Settings** If the tool has applied certain thresholds (e.g., *a cutoff distance for defining contacts or clashes*), these may be recorded in the file. These thresholds define the sensitivity of the tool in identifying interactions. These settings can be adjusted to be more or less strict depending on how fine-tuned the analysis is to be.

A good docking pose will have minimal clashes and a higher number of favorable contacts. Too many clashes indicate that the docking pose may be unrealistic, whereas a strong set of contacts suggests that the ligand is likely to bind well to the protein. The contacts list helps pinpoint which residues in the protein are critical for taurine's binding. These binding residues could be the target for future mutagenesis or *in vivo* validation studies. If the docking pose shows few clashes and strong contacts, it suggests that the taurine-protein complex is stable and that taurine may

effectively interact with the target protein. By analyzing these outputs, you can refine docking results and gain insights into how taurine interacts with its potential targets, guiding further experimental steps.

2.2.1 Hydrogen Bond Constraints

Hydrogen bond constraints were used to refine the search for favorable binding poses and improve the accuracy of docking results. Two constraints were put in place:

Distance Constraint

The maximum distance between the hydrogen atom of one molecule and the acceptor atom (*oxygen, nitrogen, or sulfur*) of the other molecule for a hydrogen bond to be considered was set to 0.4 Å. A distance of 0.4 Å is a typical cutoff value for hydrogen bonds.

Angle Constraint

The maximum angle between the hydrogen atom donor, the acceptor atom, and the heavy atom (*carbon, nitrogen, or oxygen*) connected to the acceptor atom was set to 20°. A hydrogen bond is typically considered to have an angle of around 180°, but deviations of up to 20° are often allowed.

2.3 In vivo Retinal Ischemia/Reperfusion Model

2.3.1 Chemicals

All chemicals were obtained from Sigma/Aldrich, Germany unless otherwise specified.

2.3.2 Animals

Male Wistar rats (250-300g; 8 weeks old) were housed in controlled conditions with free access to food and water, maintained on a 12-hour light/dark cycle at an environmental temperature of 20-25°C. The experiments were conducted at the Experimental Facility EL42-BIO/exp-03 (*Laboratory of Pharmacology, Department of Medicine, University of Thessaly*). Four animals were placed per cage throughout the study. The animals were familiarized with the researchers through daily handling sessions of 10 minutes each, over a period of 5 consecutive days, prior to the start of the experimental procedure. The protocol was approved by the Department of Veterinary Services of Thessaly (*License Number: 150492/26-03-2024*) and the Internal Ethics Committee of the Department of Medicine (*Approval No.: 619/10-06-2024*). All procedures adhered to Greek and European legislation.

2.3.3 Anesthesia and Eye Preparation

At the beginning of the experiments, anesthesia was induced via intraperitoneal administration of a combination of 15mg/kg tiletamine and 15mg/kg zolazepam ([Zoletil®](#); Virbac), along with 5mg/kg xylazine hydrochloride ([Rompun®](#); Bayer). To prepare the eyes, tetracaine (0.5% [tetracaine hydrochloride](#); Cooper AE) was applied topically for local anesthesia, and phenylephrine (5% [phenylephrine hydrochloride](#); Cooper AE) was used to induce mydriasis. Following the ischemia/reperfusion injury, [tobramycin](#) eye drops (0.3%; IKOBEL) were applied to prevent potential infections.

2.3.4 Study Design

The animals were randomly allocated to four experimental groups (Groups 1–4, N=6 animals/group) to investigate the effects of taurine on retinal ischemia/reperfusion injury at two time points: 24 hours (Groups 1 and 2) and 7 days (Groups 3 and 4) following retinal reperfusion. The treatment protocol for each group was as follows:

GROUPS 1 & 3 (*Ischemia/reperfusion injury*): Retinal ischemia/reperfusion injury was induced in one of the eyes of these animals by elevating intraocular pressure (IOP) for one hour while the contralateral eye was used as control (Ctrl). The animals were euthanized 24 hours [**Group 1**; control animals with one untreated eye (**Ctrl-Ctrl_24h**) and one ischemic/reperfused eye (**Ctrl-IOP_24h**)] or 7 days [**Group 3**; control animals with one untreated eye (**Ctrl-Ctrl_7days**) and one ischemic/reperfused eye (**Ctrl-IOP_7days**)] after reperfusion.

GROUPS 2 & 4 (*Ischemia/reperfusion injury + Taurine*): These animals received taurine (TCI, A0295/500) for two weeks before ischemia/reperfusion injury was induced in one of their eyes while the contralateral eye served as a control for taurine's effects. Taurine was administered *per os* through the drinking water at a concentration of 0.2M, based on the protocol by Froger et al. (2012)^[87]. Taurine administration continued throughout the experimental period until the animals were euthanized 24 hours [**Group 2**; taurine-treated animals with one untreated eye (**Tau-Ctrl_24h**) and one ischemic/reperfused eye (**Tau-IOP_24h**)] or 7 days [**Group 4**; taurine-treated animals with one untreated eye (**Tau-Ctrl_7days**) and one ischemic/reperfused eye (**Tau-IOP_7days**)] after reperfusion.

2.3.5 *In vivo* Retinal Ischemia/Reperfusion Injury by Transient Increase in Intraocular Pressure

Retinal ischemia/reperfusion injury was induced by increasing the intraocular pressure, following a modified protocol from Marín-Prida et al. (2012)^[149]. Briefly, in fully anesthetized rats, the anterior chamber of one eye was cannulated with a 30G needle attached to a PE-10 polyethylene tube connected to a saline reservoir. The reservoir was elevated to 147cm above the animal to increase the intraocular pressure to 110mmHg for 60 minutes, inducing retinal ischemia. Retinal ischemia during the experiment and tissue reperfusion at the end of the experiment were confirmed by fundus whitening and fundus blood reperfusion observation, respectively.

The same procedure was performed in the contralateral eye without elevating the saline reservoir, serving as a control. As mentioned above, the eyes were allowed to reperfusion for 24 hours (Groups 1 and 2) or 7 days (Groups 3 and 4) before the animals were euthanized by decapitation under anesthesia with intraperitoneal administration of Zoletil®.

Following that, the eyes were dissected, and the retinas were either (1) frozen at -80°C for Western blot analysis or (2) flat mounted on filter paper, fixed in 4% paraformaldehyde in 0.1M PBS for 30min, washed 2 times for 15min in 0.1M PBS and transferred in anti-freeze cryoprotectant solution^{*,[150],[151]} at -20°C for immunohistochemistry.

2.4 Immunohistochemistry

Immunohistochemistry (IHC) is a technique that relies on the specific binding of antibodies to antigens to detect and identify proteins within tissues. Immunofluorescence is a subtype of IHC where antibodies are labeled with fluorescent dyes, allowing for visualization under a standard fluorescence or confocal microscope.

In this study, the entire retina was prepared as a flat mount and immunofluorescent stained. Tissues were rinsed three times for 10 minutes each with 0.1M PBS (137mM NaCl, 2.7mM KCl, 10mM Na₂HPO₄, 1.8mM KH₂PO₄, pH 7.2-7.4). They were then incubated in 0.1M PBS containing 1% Bovine serum albumin (BSA), 0.5% (v/v) Triton X-100, 0.025% NaN₃, and the primary antibodies (as listed in [Table 3](#)) for 72 hours at 4°C with continuous shaking. The secondary antibodies ([Table 3](#)) were diluted in 0.1M PBS, and all procedures involving these antibodies were conducted in the dark to protect against photobleaching. The tissues were incubated for 16-18 hours at 4°C with the

* One liter of cryoprotectant solution consists of 500ml 0.1M PBS (50% v/v; 1.59g NaH₂PO₄ H₂O, 5.47g Na₂HPO₄ and 9.0g NaCl in 500ml distilled water; pH 7.2), 300g sucrose (30% w/v), 10g polyvinylpyrrolidone (1% w/v; PVP-40), 300ml ethylene glycol (30% v/v), with the final volume adjusted to 1L with distilled water.

secondary antibodies under constant shaking. Samples were then treated with 40 mM DAPI (4',6-diamidino-2-phenylindole) in 0.1M PBS for 30 minutes at room temperature and rinsed twice for 10 minutes with 0.1M PBS. Finally, each tissue sample was placed on a microscope slide, which was stored at 4°C until observation under a confocal microscope.

Table 3: Antibodies used in the immunohistochemical studies

Antibody	[C] _{final}
Primary antibody	
<i>Rabbit anti-NeuN</i> (MABN140 ; Sigma/Aldrich, Germany)	1:400
<i>Mouse anti-GFAP</i> (MAB360 ; Sigma/Aldrich, Germany)	1:500
Secondary antibody	
<i>CF594 Goat anti-Mouse IgG (H+L)</i> (20110 ; Biotium)	1:500
<i>CF488A Goat anti-Rabbit IgG (H+L)</i> (20012 ; Biotium)	1:500

2.5 Image Analysis

The analysis of fluorescence microscopy images was specifically aimed at studying potential astrogliosis by quantifying key features of astrocytes, including the number of branches, junctions, and endpoints. This quantitative assessment was performed using [ImageJ](#) software^[152]. The main steps involved in the analysis are outlined below:

1. File > Open: *Load the fluorescence microscopy image.*
2. Image > Color > Split Channels: *Separate the image into its color channels.*
3. Work with Red Channel: *Focus on the red channel, where the astrocyte marker GFAP is visualized.*
4. Image > Adjust > Brightness/Contrast: *Optimize the image for better visibility of features, then click OK.*
5. Process > Make Binary: *Convert the image into a binary format, isolating the structures of interest.*
6. Process > Make Binary > Skeletonize: *Reduce the binary image to its skeletal form, highlighting the branching patterns of astrocytes.*
7. Process > Filters > Maximum 3D: *Apply 3D maximum filters to enhance the skeletal representation.*
8. Process > Make Binary > Skeletonize: *Repeat the skeletonization to refine the image further.*

9. Analyze > Skeleton > Analyze Skeleton (2D/3D): *Finally, conduct a comprehensive analysis of the skeletal structure, obtaining measurements of branches, junctions, and endpoints.*

2.6 Statistical Analysis

Statistical analysis was conducted using [Jamovi 2.3.28](#) to assess whether taurine affects astrogliosis, measured by the number of branches, junctions, and endpoints of astrocytes, at 24 hours and 7 days following retinal ischemia/reperfusion injury (see: [2.5 Image Analysis](#)). A two-way ANOVA was used to evaluate the interaction effects of treatment (*taurine vs. control*) and time (*24 hours vs. 7 days*). Additionally, one-way ANOVA was applied to compare differences between the four groups (*Ctrl-Ctrl, Ctrl-IOP, Tau-Ctrl, Tau-IOP*) at both time points (see: [2.3.4 Study Design](#)).

3. RESULTS

3.1 *In silico* Identification and Validation of Taurine Targets in Ischemia/Reperfusion Injury

3.1.1 Common Targets of Taurine and Ischemia/Reperfusion Injury

The potential targets of taurine were identified using nine databases and computational tools (*see: 2.1.1 Identification of Possible Taurine Targets*), resulting in 2,097 taurine-related genes ([Appendix I](#)). Similarly, the potential retina ischemia/reperfusion-associated targets were sourced from three databases (*see: 2.1.2 Identification of Possible Ischemia/Reperfusion Injury-Associated Targets*), yielding 1,991 target genes ([Appendix II](#)). The "Draw Venn Diagram" online tool was employed to identify potential therapeutic targets for taurine in ischemia/reperfusion (I/R) injury. This analysis revealed 419 common genes that are both associated with taurine and ischemia/reperfusion injury ([Figure 12](#), [Table 4](#)).

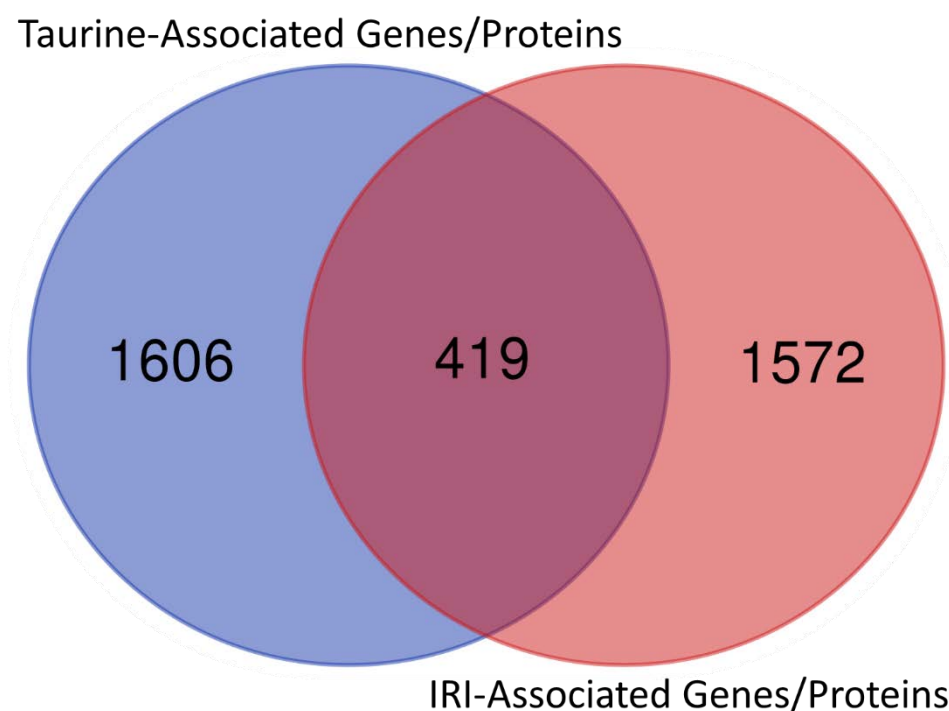


Figure 12. Intersection of Taurine-Related Genes/Proteins and Ischemia/Reperfusion-Associated Genes/Proteins. The Venn diagram shows the overlap between taurine-related genes/proteins and those associated with ischemia/reperfusion.

Table 4: Genes/proteins associated with both taurine and ischemia/reperfusion injury

ABAT	BAD	CDKN2A	EED	GLUD1	IDH1	LRP6	NOS3	PTGER3	SQSTM1
ABCC1	BAK1	CEBPA	EGF	GLUL	IDO1	LTF	NOTCH1	PTGER4	SRC
ACACA	BAX	CFP	EGFR	GP1BA	IFNG	MALT1	NOX4	PTGS2	STAT3
ACE	BCHE	CHAT	EGLN1	GPBAR1	IGF1R	MAP1LC3B	NPY1R	PTK2	TAC1
ACHE	BCL2	CHRM1	EHMT1	GPT	IGFBP3	MAP3K11	NQO1	PTPN1	TACR1
ACOD1	BCL2L1	CHRM2	ELA2	GPX1	IKBKG	MAPK1	NR3C1	PTPN11	TBXA2R
ACTA2	BCL2L11	CHRM3	ELANE	GRIN1	IKZF1	MAPK14	NR3C2	RAF1	TBXAS1
ADA	BDKRB2	CHUK	ENG	GRIN2A	IL10	MAPK3	OGA	RELA	TEK
ADAM17	BDNF	CNDP1	ENO2	GRIN2B	IL15	MAPK8	OPRD1	REN	TF
ADK	BID	CNR1	EPHX1	GRM2	IL18	MAPK9	OPRK1	ROBO1	TGFB1
ADORA1	BIRC5	CNR2	EPHX2	GRM5	IL18R1	MAPKAPK2	OPRM1	RPS27A	TGM3
ADORA2A	BMP2	COL18A1	EPOR	GRM7	IL1B	MAPT	P4HB	RUNX1	TH
ADORA2B	BMPR2	COMT	ERN1	GSDMD	IL2	MBP	PADI4	S1PR2	THBD
ADORA3	BPI	CPB2	ESR1	GSK3B	IL6	MCU	PARP1	SCML2	TIMP1
ADRB1	BRD4	CPT1A	ESR2	GSR	IL8RA	MDH2	PARP2	SDHA	TKT
ADRB2	BTG2	CREB1	F10	GUCY1A1	IL8RB	MDM2	PCNA	SELP	TLR4
ADRB3	C1S	CTLA4	F11	HAMP	INS	MICU2	PCSK9	SERPINE1	TNC
AGT	C4A	CTSB	F13A1	HBB	INSR	MIF	PDE4A	SERPINI1	TNF
AGTR1	CA1	CTSD	F2	HCRT	IRS1	MMP1	PDE5A	SI	TNFRSF12A
AGTR2	CA2	CTSL	FABP2	HCRT1	ITGA2B	MMP13	PDE9A	SIGMAR1	TNFSF13B
AKR1B1	CA9	CX3CL1	FAS	HDAC1	ITGA4	MMP14	PEPD	SIRT1	TNFSF9
AKT1	CACNA1B	CXCL12	FASN	HDAC6	ITGA5	MMP2	PIK3CD	SIRT2	TNNI3
AKT1S1	CACNA1C	CXCL13	FCGR3A	HGF	ITGAV	MMP8	PIK3R1	SIRT3	TP53
ALDH2	CALCRL	CXCR1	FCGR3B	HIF1A	ITGB1	MMP9	PIM1	SIRT6	TP53BP2
ALDH5A1	CALM1	CXCR2	FCN2	HMGB1	ITGB3	MPO	PINK1	SLC12A5	TPH1
ALOX15	CAPN1	CXCR3	FFAR2	HMGCR	JAK2	MTA1	PKLR	SLC18A2	TRAP1
ANGPT2	CASP1	CXCR4	FGF1	HMOX1	JMJD6	MTOR	PLA2G10	SLC1A2	TRPV1
AOC1	CASP3	CYBB	FGF19	HMOX2	JUN	NAMPT	PLAU	SLC29A1	TSPO
APAF1	CASP8	CYCS	FLNA	HP	KAT2B	NCAM1	PLG	SLC2A1	TYK2
APLNR	CASP9	CYP2E1	FLRT3	HRH1	KCNJ8	NCAM2	PNP	SLC5A1	TYR
APOA1	CASR	CYSLTR1	FN1	HRH2	KCNN3	NCF1	POLB	SLC5A2	UGT1A1
APOE	CAT	DAO	FOS	HRH3	KDM1A	NDRG4	PPARA	SLC6A1	UMOD
APP	CBS	DBH	FOXO1	HSD17B14	KDR	NDUFC2	PPARG	SLC6A11	USP28
ARG1	CCL3	DDAH1	FYN	HSP90AA1	KEAP1	NDUFS4	PPARGC1A	SLC6A13	USP7
ARG2	CCL5	DDIT3	G6PC1	HSP90AB1	KRIT1	NEU1	PPIA	SLC6A2	VCAM1
ATF2	CCR2	DEFA1	G6PD	HSPA5	LAMTOR5	NFKB1	PRDX5	SLC6A3	VCP
ATG7	CCR4	DLD	GAD1	HSPB1	LCN2	NFKBIA	PRKACA	SLC6A4	VDR
ATM	CCR5	DPP4	GC	HTR2A	LDHA	NGF	PRKCA	SLC9A1	VEGFA
ATP2A2	CD55	DPYSL2	GCH1	HTR2B	LDLR	NLRP3	PRKCD	SMPD1	XDH
ATP7A	CD69	DRD2	GCK	HTR3A	LEPR	NLRX1	PRKDC	SNAP25	XIAP
AXL	CD74	DUSP1	GGT1	HTR4	LGALS3	NOS1	PRKN	SNCA	YWHAB
B2M	CD80	EDNRA	GLO1	ICAM1	LINGO1	NOS2	PRMT5	SPON1	

3.1.2 Protein-Protein Interaction Networks of the Common Targets of Taurine and Ischemia/Reperfusion Injury and Identification of Core Targets

The common targets of taurine and I/R injury were uploaded to the STRING webtool to construct a Protein–Protein Interaction (PPI) networks ([Figure 13](#)). These PPI networks were then imported into Cytoscape software, where the cytoHubba plugin was used to identify key hubs and significant nodes (core targets) within the biological networks. The top 15 common taurine and I/R injury targets identified through the Degree, Maximum Neighborhood Component (MNC), Maximal Clique Centrality (MCC), and Closeness ranking methods ([Table 5](#)) were subsequently analyzed using the Draw Venn Diagram webtool to identify unique molecules. The analysis revealed 20 unique molecules across all four ranking methods ([Table 6](#)). These unique molecules (core targets) were further validated using molecular docking to predict how taurine might bind to them (*see: [3.1.4](#)*).

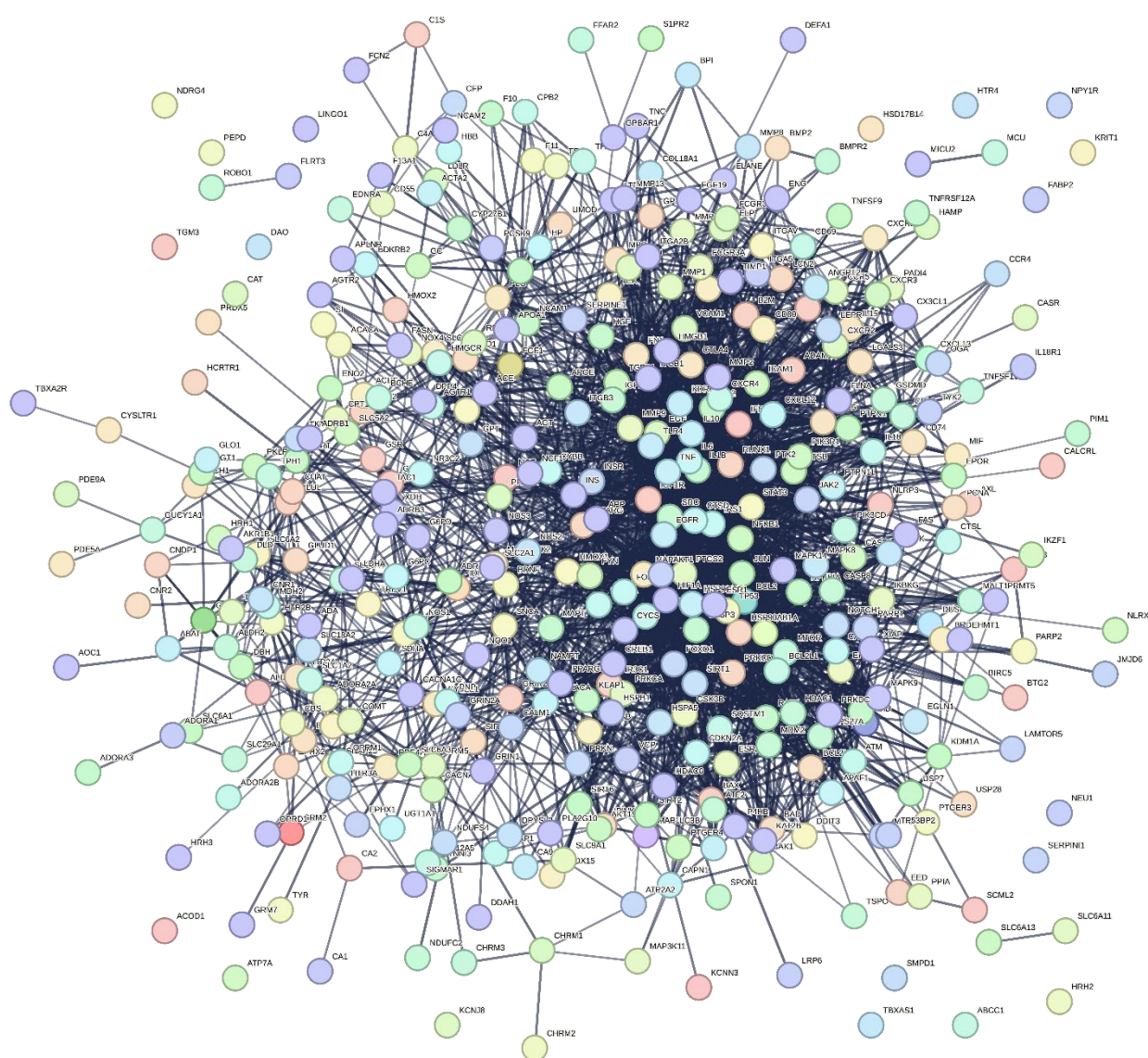


Figure 13: Protein–Protein Interaction network of common targets of taurine and I/R injury. [String Network](#) with minimum required interaction score: 0.7 (high confidence)

Table 5: Top 15 taurine-IRI targets identified through the Degree, Maximum Neighborhood Component (MNC), Maximal Clique Centrality (MCC), and Closeness ranking methods

Degree	MNC	MCC	Closeness
INS	INS	TNF	TNF
CASP3	CASP3	CASP3	CASP3
TNF	TNF	IL6	INS
IL6	IL6	JUN	IL6
JUN	JUN	BCL2	JUN
BCL2	BCL2	NFKB1	BCL2
HSP90AA1	HSP90AA1	FOS	TLR4
TLR4	TLR4	PTGS2	NFKB1
NFKB1	NFKB1	MAPK3	SRC
SRC	SRC	NFKBIA	MAPK3
STAT3	STAT3	STAT3	STAT3
TP53	TP53	TP53	TP53
EGFR	EGFR	IFNG	IL1B
IL1B	IL1B	IL1B	EGFR
AKT1	AKT1	AKT1	AKT1

Table 6: Core taurine-IRI targets

AKT1	FOS	IL6	NFKB1	STAT3
BCL2	HSP90AA1	INS	NFKBIA	TLR4
CASP3	IFNG	JUN	PTGS2	TNF
EGFR	IL1B	MAPK3	SRC	TP53

These 20 core targets were introduced in the SIGnaling network open resource 3.0 (SIGNOR 3.0). The interactions with a score > 0.9 between the 20 core targets are presented in [Figure 14](#) and detailed in [Table 7](#).

Table 7. Interactions between core targets. SIGNOR 3.0; score > 0.90.

Source ID	Source Name	Source Category	Target ID	Target Name	Target Category	Effect
P31749	AKT1	protein	SIGNOR-PF27	FOXO	Protein family	<i>inhibition</i>
P31749	AKT1	protein	O43524	FOXO3	protein	<i>inhibition</i>
P05231	IL6	protein	P08887	IL6R	protein	<i>activation</i>
Q92793	CREBBP	protein	P04637	TP53	protein	<i>activation</i>
P00533	EGFR	protein	P29353	SHC1	protein	<i>activation</i>
P12931	SRC	protein	O60716	CTNND1	protein	<i>activation</i>
P01375	TNF	protein	P19438	TNFRSF1A	protein	<i>activation</i>
O14920	IKBKB	protein	P25963	NFKBIA	protein	<i>inhibition</i>
P42345	MTOR	protein	P31749	AKT1	protein	<i>activation</i>
P00533	EGFR	protein	P62993	GRB2	protein	<i>none</i>
P12931	SRC	protein	P46937-3	YAP1	protein	<i>activation</i>
P01584	IL1B	protein	P14778	IL1R1	protein	<i>activation</i>
P31749	AKT1	protein	P42345	MTOR	protein	<i>activation</i>
P01375	TNF	protein	P20333	TNFRSF1B	protein	<i>activation</i>
P05412	JUN	protein	SIGNOR-C154	AP1	complex	<i>complex-formation</i>
P04637	TP53	protein	SIGNOR-C6	CBP/p300	complex	<i>activation</i>
P01100	FOS	protein	SIGNOR-C154	AP1	complex	<i>complex-formation</i>
P98170	XIAP	protein	P42574	CASP3	protein	<i>inhibition</i>
P45983	MAPK8	protein	P05412	JUN	protein	<i>activation</i>
Q00987	MDM2	protein	P04637	TP53	protein	<i>inhibition</i>
P04637	TP53	protein	Q00987	MDM2	protein	<i>transcription-activation</i>
P01133	EGF	protein	P00533	EGFR	protein	<i>activation</i>
Q09472	EP300	protein	P04637	TP53	protein	<i>activation</i>
P01100	FOS	protein	P05412	JUN	protein	<i>activation</i>
P12931	SRC	protein	P46940	IQGAP1	protein	<i>activation</i>
P31749	AKT1	protein	Q96GX5	MASTL	protein	<i>activation</i>
P12931	SRC	protein	P29474	NOS3	protein	<i>activation</i>
P12931	SRC	protein	O14744	PRMT5	protein	<i>inhibition</i>
P31749	AKT1	protein	P51812	RPS6KA3	protein	<i>inhibition</i>
P12931	SRC	protein	P11413	G6PD	protein	<i>activation</i>
P12931	SRC	protein	P54646	PRKAA2	protein	<i>inhibition</i>
P31749	AKT1	protein	Q00613	HSF1	protein	<i>activation</i>
P27361	MAPK3	protein	Q99814	EPAS1	protein	<i>activation</i>
P27361	MAPK3	protein	Q8TB45	DEPTOR	protein	<i>activation</i>
Q13237	PRKG2	protein	P00533	EGFR	protein	<i>inhibition</i>
P31749	AKT1	protein	Q92900	UPF1	protein	<i>activation</i>
P12931	SRC	protein	P17655	CAPN2	protein	<i>activation</i>
Q13188	STK3	protein	P40763	STAT3	protein	<i>inhibition</i>
P12931	SRC	protein	P50570	DNM2	protein	<i>inhibition</i>
P49841	GSK3B	protein	P12931	SRC	protein	<i>inhibition</i>
Q15185	PTGES3	protein	P07900	HSP90AA1	protein	<i>activation</i>
P31948	STIP1	protein	P07900	HSP90AA1	protein	<i>inhibition</i>
O15151	MDM4	protein	P04637	TP53	protein	<i>inhibition</i>
P01308	INS	protein	P06213	INSR	protein	<i>activation</i>

3.1.3 Enrichment Analysis

KEGG and GO enrichment analyses help interpret large datasets and uncover potential mechanisms of action related to drug effects. The [Metascape](#) platform was used to perform KEGG and GO enrichment analyses^[145]. The taurine and ischemia/reperfusion injury overlapping targets were uploaded to the platform, with the following parameters: Min Overlap = 3, P-Value Cutoff = 0.01, and Min Enrichment = 1.5. After the enrichment analyses, the clustering network was exported and categorized according to the P-value. The top 20 enriched ontology clusters, regardless their origin (*GO/KEGG terms, canonical pathways, hall mark gene sets, etc.*) are presented in [Figure 15](#). Of the top 20 recognized clusters 11 are associated with IRI.

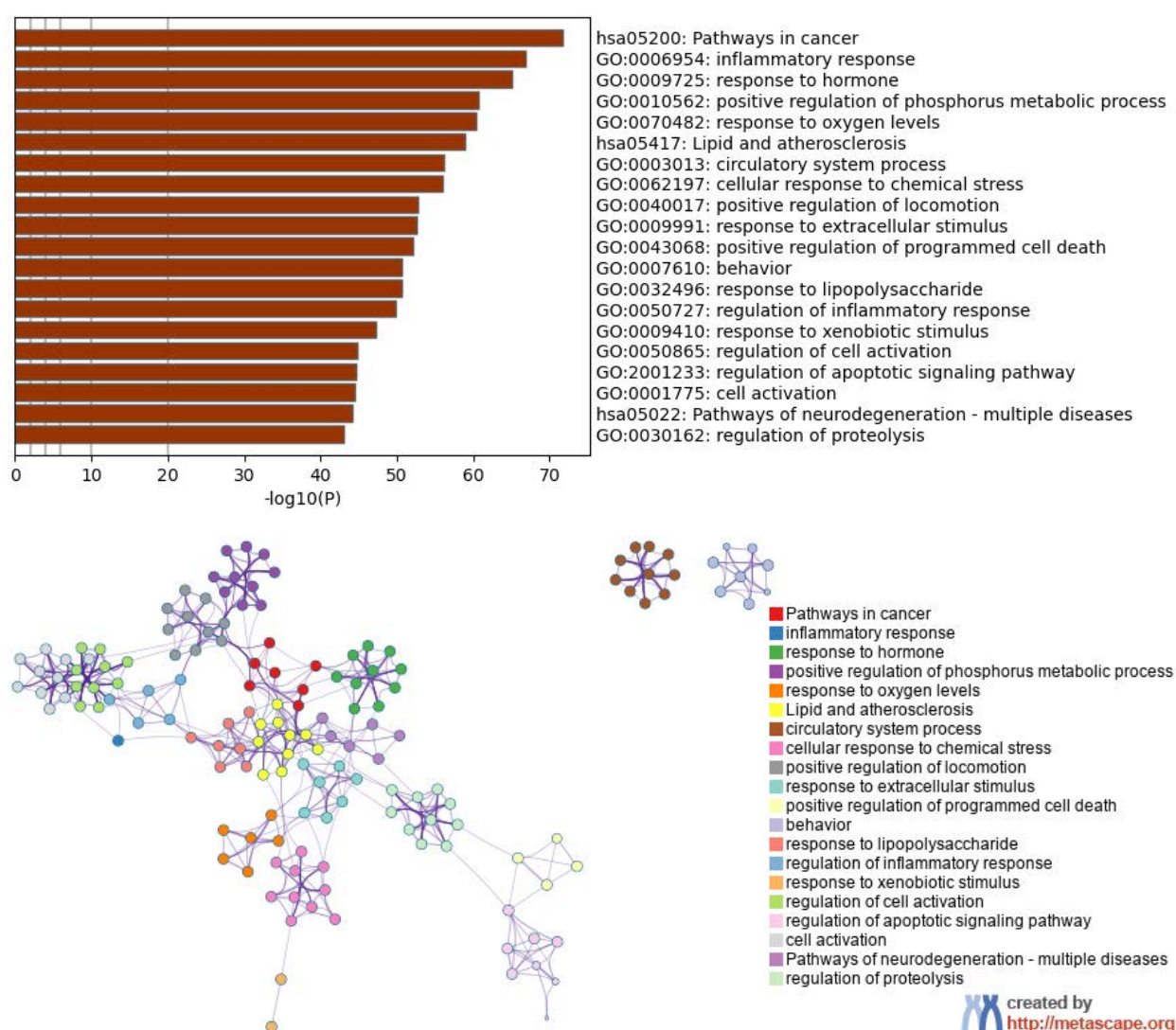


Figure 15: Top 20 enriched Ontology Clusters *Above:* Bar graph of enriched terms across taurine and ischemia/reperfusion injury overlapping targets lists by p-values. *Below:* representative terms from the full cluster converted them into a network layout. Each term is represented by a circle node, where its size is proportional to the number of input genes fall under that term, and its color represents its cluster identity (i.e., nodes of the same color belong to the same cluster). Terms with a similarity score > 0.3 are linked by an edge (the thickness of the edge represents the similarity score). One term from each cluster is selected to have its term description shown as label.

The figures below display the 20 most substantially enriched GO terms and the top 20 enriched KEGG pathways (*excluding cancer, parasite, and infection-related pathways*) with a p-value of less than 0.01. GO enrichment analysis was divided into Biological Processes ([Figure 16](#)), Molecular Functions ([Figure 17](#), [Table 8](#)) and Cellular Components ([Figure 18](#)). In the cases of Cellular Components, and Molecular Functions the total results were less than 20 and all were retained.

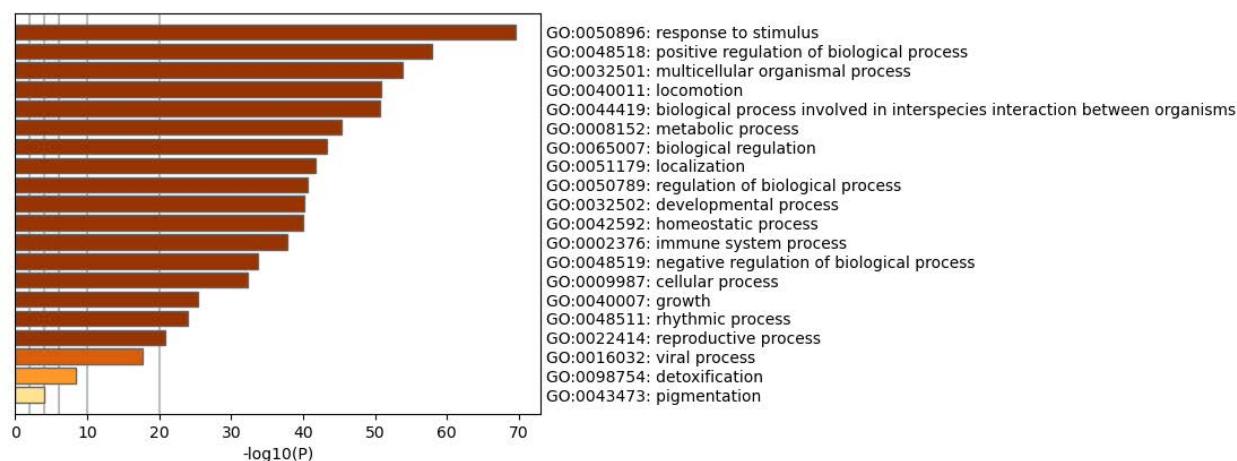


Figure 16: GO Biological Processes. *The top 20 biological pathways influenced by common taurine and ischemia/reperfusion injury targets.*

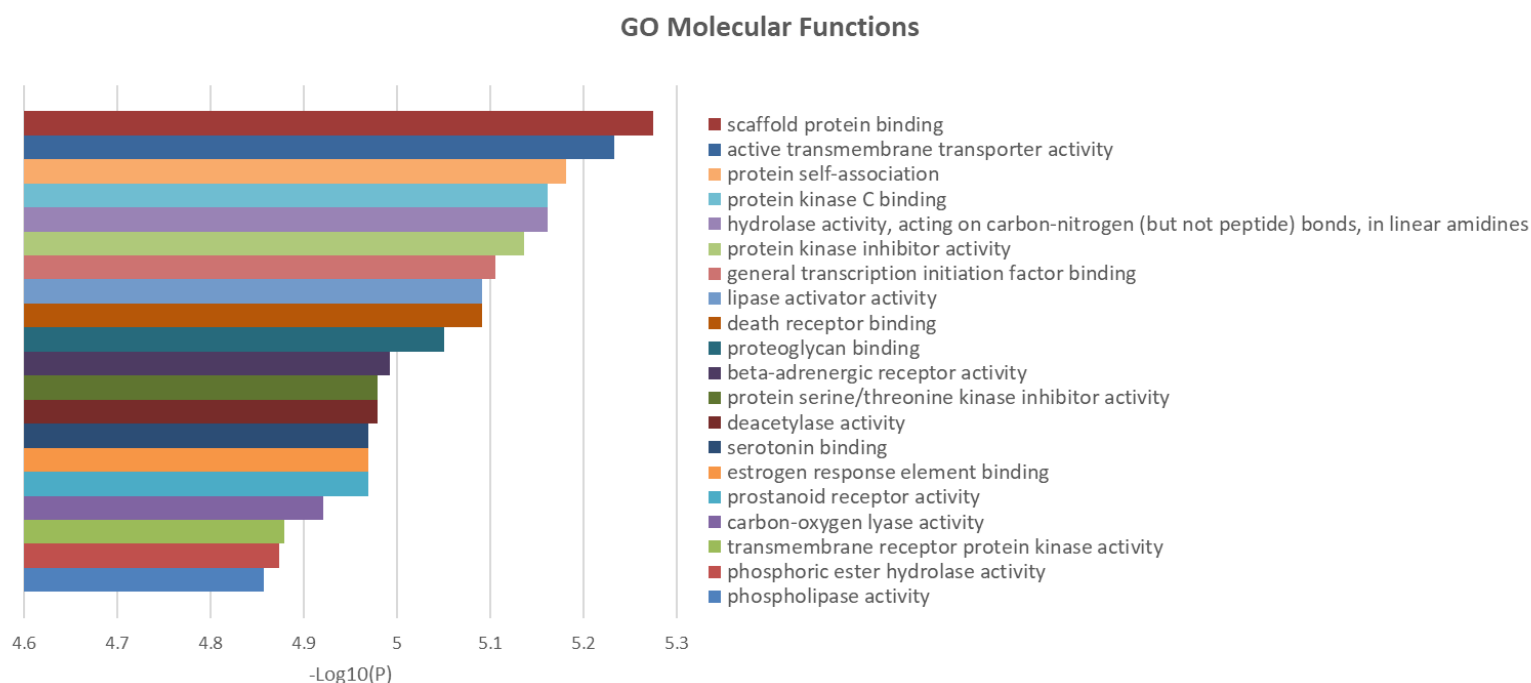


Figure 17: GO Molecular Functions. *Molecular functions influenced by common taurine and ischemia/reperfusion injury targets.*

Table 8: GO Molecular Functions. *Molecular functions influenced by common taurine and ischemia/reperfusion injury targets sorted by p-value and involved genes/proteins*

Description	LogP	Involved Targets
scaffold protein binding	-5,27440	CASP8 CHUK FYN GRM2 HSP90AA1 NOS1 NOS3 SRC
active transmembrane transporter activity	-5,23314	ATP2A2 ATP7A SLC29A1 KCNJ8 ABCC1 NDUFC2 NDUFS4 SLC1A2, SLC5A1 SLC5A2 SLC6A1 SLC6A2 SLC6A3 SLC6A4 SLC6A11 SLC6A13 SLC9A1 SLC18A2 SLC12A5 MCU
protein self-association	-5,18171	ACHE MDH2 PPARG CCL5 SLC2A1 TP53 MALT1 PCSK9
protein kinase C binding	-5,16188	FLNA HSPB1 IRS1 ITGAV ITGB3 SRC SQSTM1
hydrolase activity, acting on carbon-nitrogen (<i>but not peptide</i>) bonds, in linear amidines	-5,16106	ARG1 ARG2 PADI4 DDAH1
protein kinase inhibitor activity	-5,13649	AKT1 CASP3 CDKN2A HSPB1 SNCA YWHAB KAT2B AKT1S1
general transcription initiation factor binding	-5,10548	ESR1 MTOR NR3C1 JUN NR3C2 RELA TP53
death receptor binding	-5,09140	BDNF BID CASP3 CASP8 NGF
lipase activator activity	-5,09140	CASP3 FYN CCL3 CCL5 SRC
proteoglycan binding	-5,05047	APOE CTSB CTSN FCN2 FN1 TNC
beta-adrenergic receptor activity	-4,99195	ADRB1 ADRB2 ADRB3
deacetylase activity	-4,97866	HDAC1 HDAC6 SIRT2 SIRT3 SIRT1 SIRT6
protein serine/threonine kinase inhibitor activity	-4,97866	AKT1 CASP3 CDKN2A HSPB1 KAT2B AKT1S1
prostanoid receptor activity	-4,96950	PPARG PTGER3 PTGER4 TBXA2R
estrogen response element binding	-4,96950	ESR1 ESR2 NR3C1 NR3C2
serotonin binding	-4,96950	HTR2A HTR2B HTR3A SLC6A4
carbon-oxygen lyase activity	-4,92080	CA1 CA2 CA9 CBS ENO2 FASN POLB TBXAS1
transmembrane receptor protein kinase activity	-4,87962	AXL BMPR2 EGFR ENG IGF1R INSR KDR TEK
phosphoric ester hydrolase activity	-4,87405	BDKRB2 CASR CHRM1 CHRM3 CCR5 DUSP1 EDNRA EPHX2 G6PC1 PDE4A PDE9A PTK2 PTPN1 PTPN11 CCL5 SMPD1 PDE5A
phospholipase activity	-4,85751	BDKRB2 CASR CHRM1 CHRM3 CCR5 EDNRA CCL5 SMPD1 PLA2G10

The GO enrichment analysis suggests that taurine may have therapeutic benefits on RIRI via protein serine/threonine kinase inhibitor activity, and metabolic and immune system processes, among other factors ([Figures 16-18](#), [Table 8](#)).

The KEGG pathway enrichment analysis revealed 107 pathways. The top 20 enriched pathways (excluding those related to cancer, parasites, and infections, [Figure 19](#)) indicate that taurine may play a role in RIRI through various signaling pathways including, apoptosis, neurodegeneration, PI3K-Akt signaling, TNF signaling, calcium signaling, and IL-17 signaling pathways.

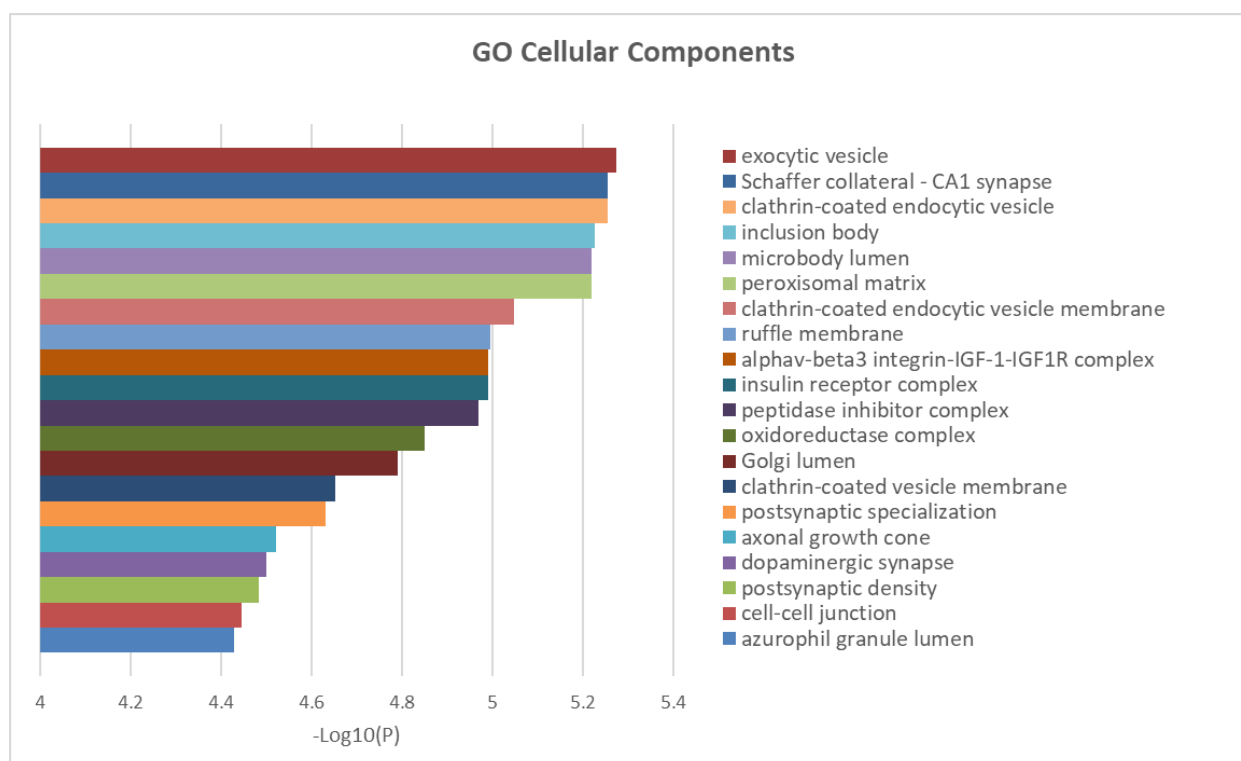


Figure 18: GO Cellular Components. Cellular components influenced by common taurine and ischemia/reperfusion injury targets.

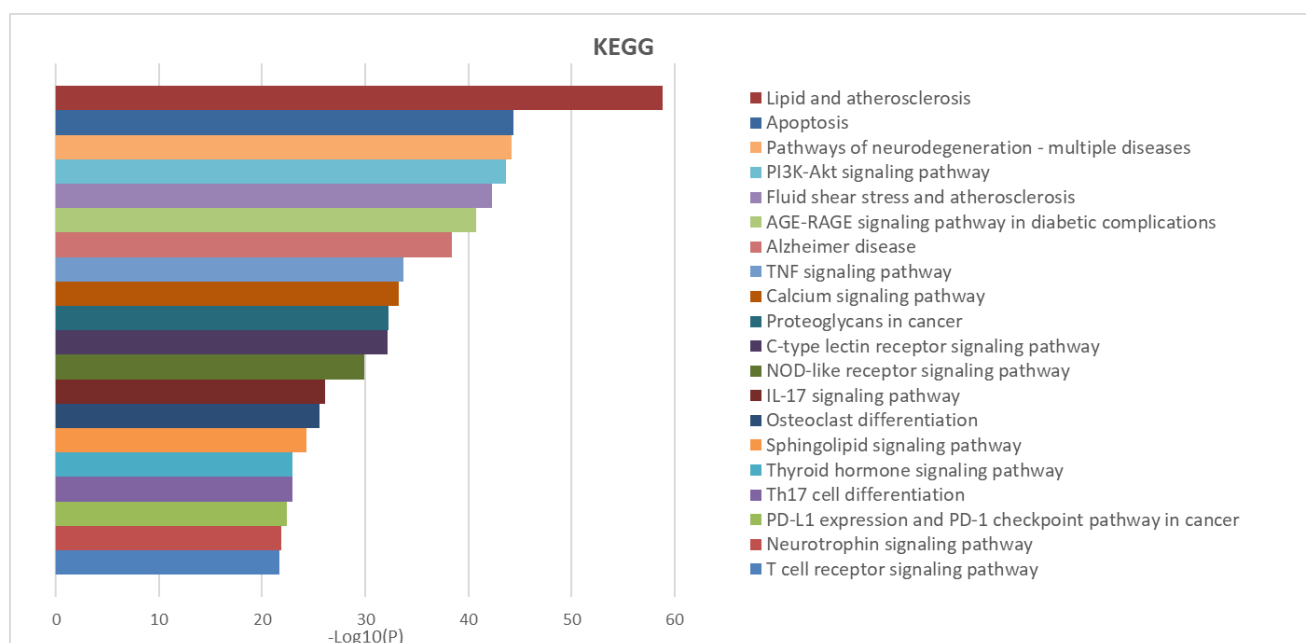


Figure 19: KEGG Enrichment Analysis. Top 20 enriched pathways (excluding those related to cancer, parasites, and infections) influenced by common taurine and ischemia/reperfusion injury targets.

3.1.4 Molecular Docking - *In silico* Validation of Taurine binding to Core Targets

The PPI network of potential common targets of taurine and I/R injury ([Figure 13](#)) was analyzed by CytoHubba, and 20 core targets were identified ([Table 6](#)). Molecular docking was used to assess the binding affinity of taurine to the 20 identified core targets ([Figure 20-24](#)) and their docking scores ([Table 9](#)).

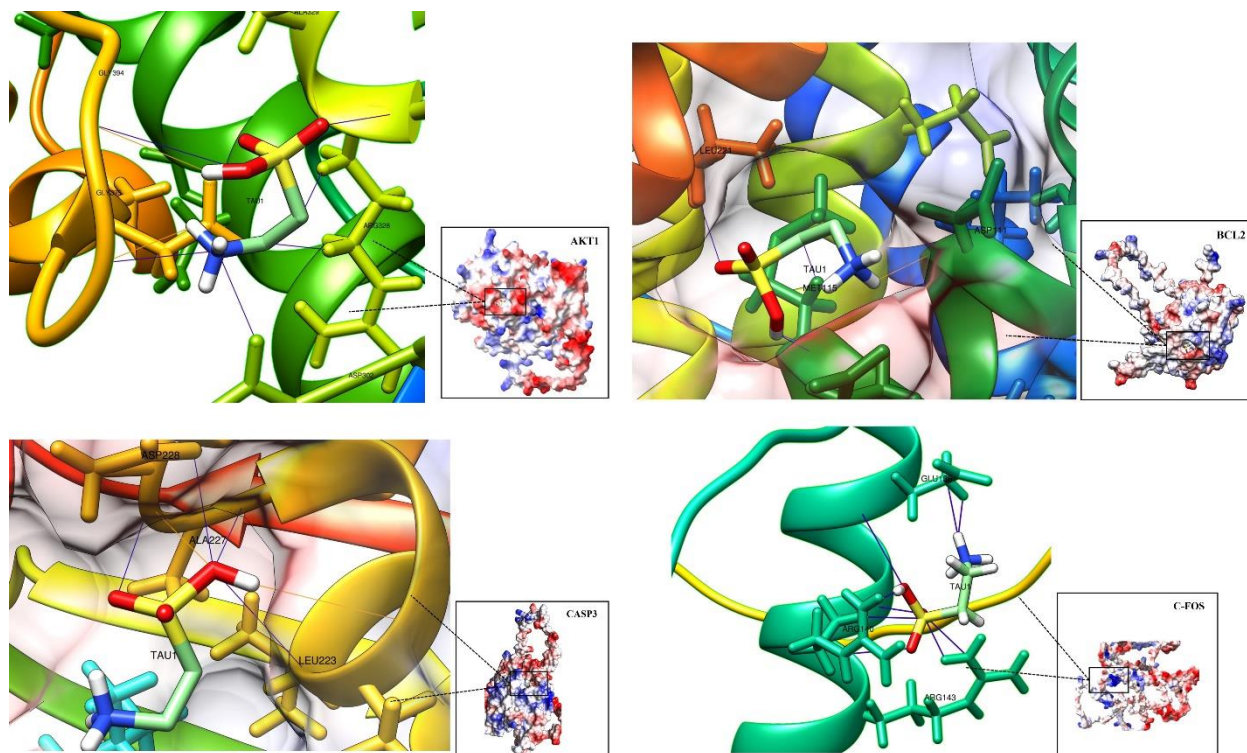


Figure 20: Taurine binding to AKT1, BCL2, CASP3 and EGFR. Orange line: Hydrogen bond; Blue line: Other pseudobonds.

AKT1

Molecular docking indicated that taurine binds to AKT1 [RAC (Rho family)-alpha serine/threonine-protein kinase] via three hydrogen bonds and strong electrostatic interactions ([Figure 20](#)). Hydrogen bonds occur between taurine and amino acids ALA 329, GLY 394, and GLY 395. The docking score is -3.9.

BCL2

Molecular docking indicated that taurine binds to BCL2 via one hydrogen bond and strong electrostatic interactions ([Figure 20](#)). Hydrogen bond occur between taurine and amino acid ASP 111. The docking score is -3.7.

CASP3

Molecular docking indicated that taurine binds to Caspase-3 via two hydrogen bonds, and strong electrostatic interactions ([Figure 20](#)). Hydrogen bonds occur between taurine and amino acids LYS 224, and ASP 228 (2 H-bonds). The docking score is -3.7.

EGFR

Molecular docking indicated that taurine binds to Epidermal Growth Factor Receptor (EGFR) via three hydrogen bonds, and strong electrostatic interactions ([Figure 20](#)). Hydrogen bonds occur between taurine and amino acids LEU 325, ASN 328, and VAL 350. The docking score is -4.7.

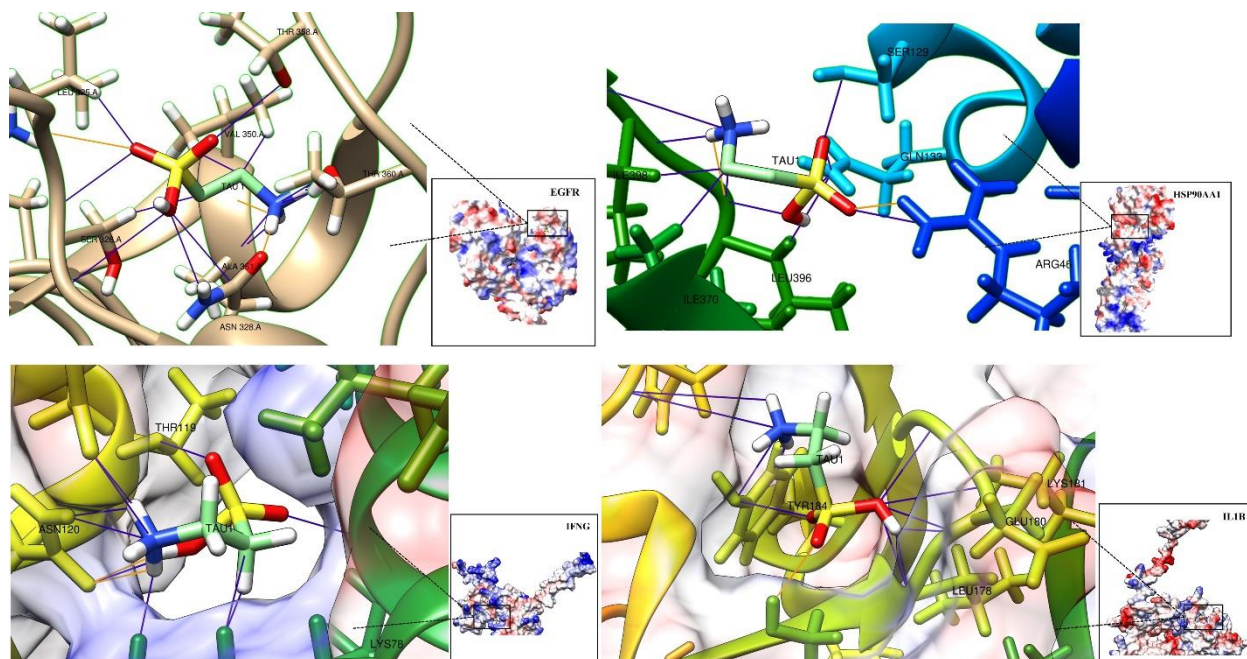


Figure 21: Taurine binding to c-FOS, HSP90AA1, IFNG and IL-1 β . Orange line: Hydrogen bond; Blue line: Other pseudobonds.

c-FOS

Molecular docking indicated that taurine binds to c-FOS via electrostatic interactions ([Figure 21](#)). The docking score is -3.0.

HSP90AA1

Molecular docking indicated that taurine binds to HSP90AA1 (Heat Shock Protein 90 Alpha Family Class A Member 1) via two hydrogen bonds, and strong electrostatic interactions ([Figure 21](#)). Hydrogen bonds occur between taurine and amino acids ARG 46, and LEU 396. The docking score is -3.9.

IFNG

Molecular docking indicated that taurine binds to interferon gamma (IFNG) via two hydrogen bonds, and strong electrostatic interactions ([Figure 21](#)). Hydrogen bonds occur between taurine and amino acid ASN 120 (2 H-bonds). The docking score is -3.3.

IL1B

Molecular docking indicated that taurine binds to interleukin-1 beta (IL-1 β) via one hydrogen bond, and strong electrostatic interactions ([Figure 21](#)). Hydrogen bond occur between taurine and amino acid SER 159. The docking score is -3.9.

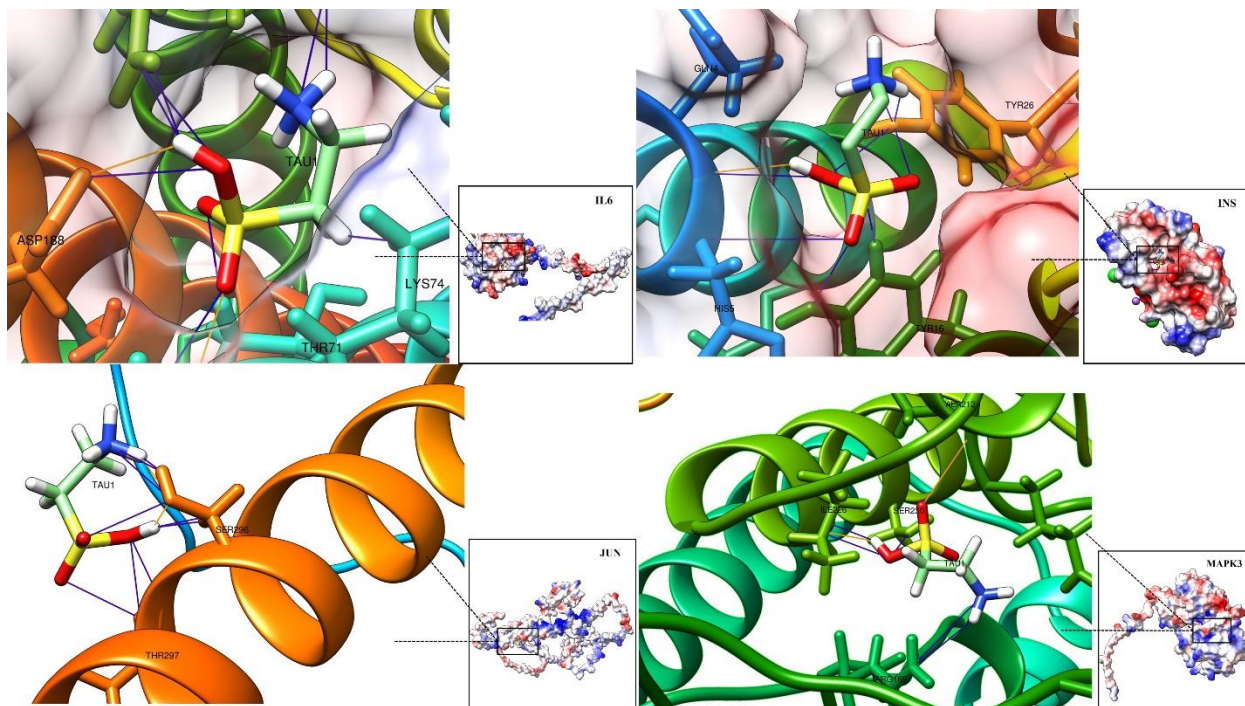


Figure 22: Taurine binding to IL-6, INS, JUN and MAPK3. Orange line: Hydrogen bond; Blue line: Other pseudobonds.

IL-6

Molecular docking indicated that taurine binds to interleukin-6 (IL-6) via two hydrogen bonds and strong electrostatic interactions ([Figure 22](#)). Hydrogen bonds occur between taurine and amino acids SER 75, and ASP 188. The docking score is -3.9.

INS

Molecular docking indicated that taurine binds to insulin (INS) via one hydrogen bond and strong electrostatic interactions ([Figure 22](#)). Hydrogen bond occurs between taurine and amino acid GLN 4 (B chain). The docking score is -3.7.

JUN

Molecular docking indicated that taurine binds to JUN via two hydrogen bonds and strong electrostatic interactions ([Figure 22](#)). Hydrogen bonds occur between taurine and amino acid SER 296 (2 H-bonds). The docking score is -2.6.

MAPK3

Molecular docking indicated that taurine binds to MAPK3 [Mitogen-Activated Protein Kinase 3 or ERK1 (Extracellular Signal-Regulated Kinase 1)] via two hydrogen bonds and strong electrostatic interactions ([Figure 22](#)). Hydrogen bonds occur between taurine and amino acids ALA 212, and ILE 226. The docking score is -4.3.

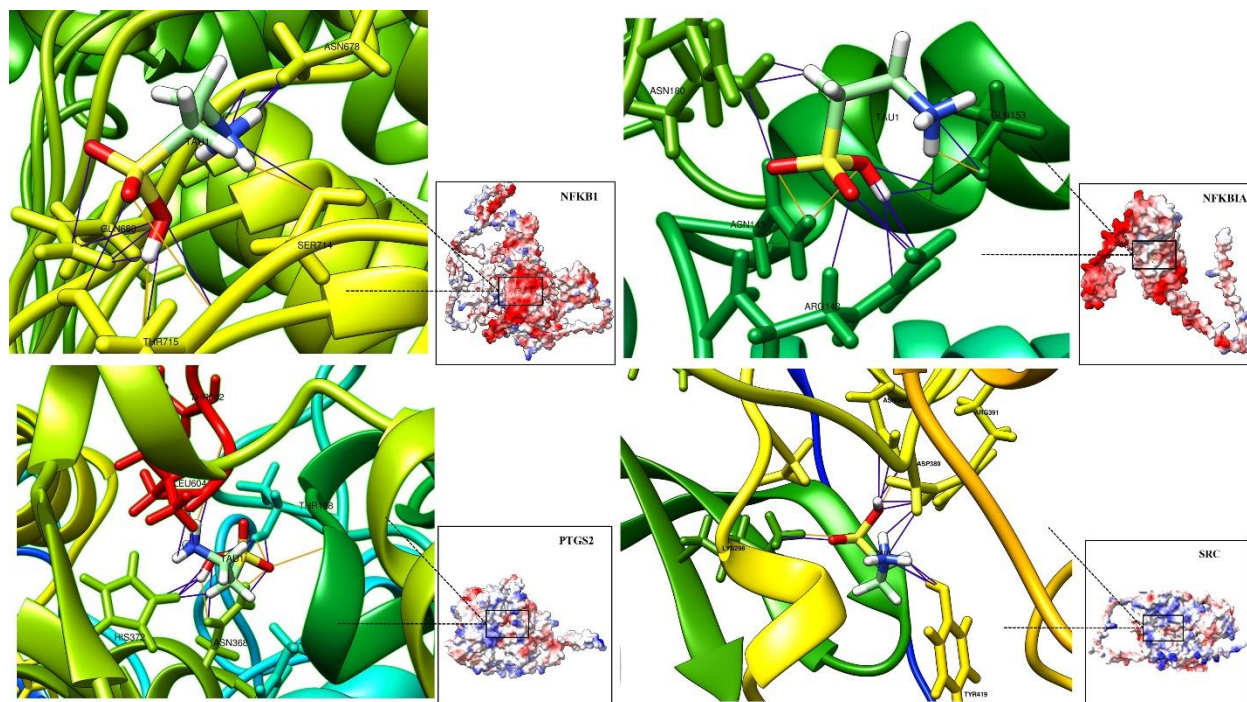


Figure 23: Taurine binding to NFKB1, NFKBIA, PTGS2, SRC and STAT3. Orange line: Hydrogen bond; Blue line: Other pseudobonds.

NFKB1

Molecular docking indicated that taurine binds to the p50 subunit of NFKB1 (Nuclear Factor Kappa B Subunit 1) via three hydrogen bonds and strong electrostatic interactions ([Figure 23](#)). Hydrogen bonds occur between taurine and amino acids SER 714, and THR 715 (2 H-bonds). The docking score is -3.5.

NFKBIA

Molecular docking indicated that taurine binds to NFKBIA (Nuclear Factor Kappa B Inhibitor Alpha) via three hydrogen bonds and strong electrostatic interactions ([Figure 23](#)). Hydrogen bonds occur between taurine and amino acids ASN 145 (2 H-bonds), GLU 153. The docking score is -3.4.

PTGS2

Molecular docking indicated that taurine binds to PTGS2 [Prostaglandin-Endoperoxide Synthase 2 or Cyclooxygenase-2 (COX-2)] via six hydrogen bonds and strong electrostatic interactions

([Figure 23](#)). Hydrogen bonds occur between taurine and amino acids THR 602, THR 198 (2 H-bonds), ASN 168, and ASN 368 (2 H-bonds). The docking score is -4.5.

SRC

Molecular docking indicated that taurine binds to Src tyrosine kinase via two hydrogen bonds, and strong electrostatic interactions ([Figure 23](#)). Hydrogen bonds occur between taurine and amino acids LYS 298, and ASN 394. The docking score is -3.9.

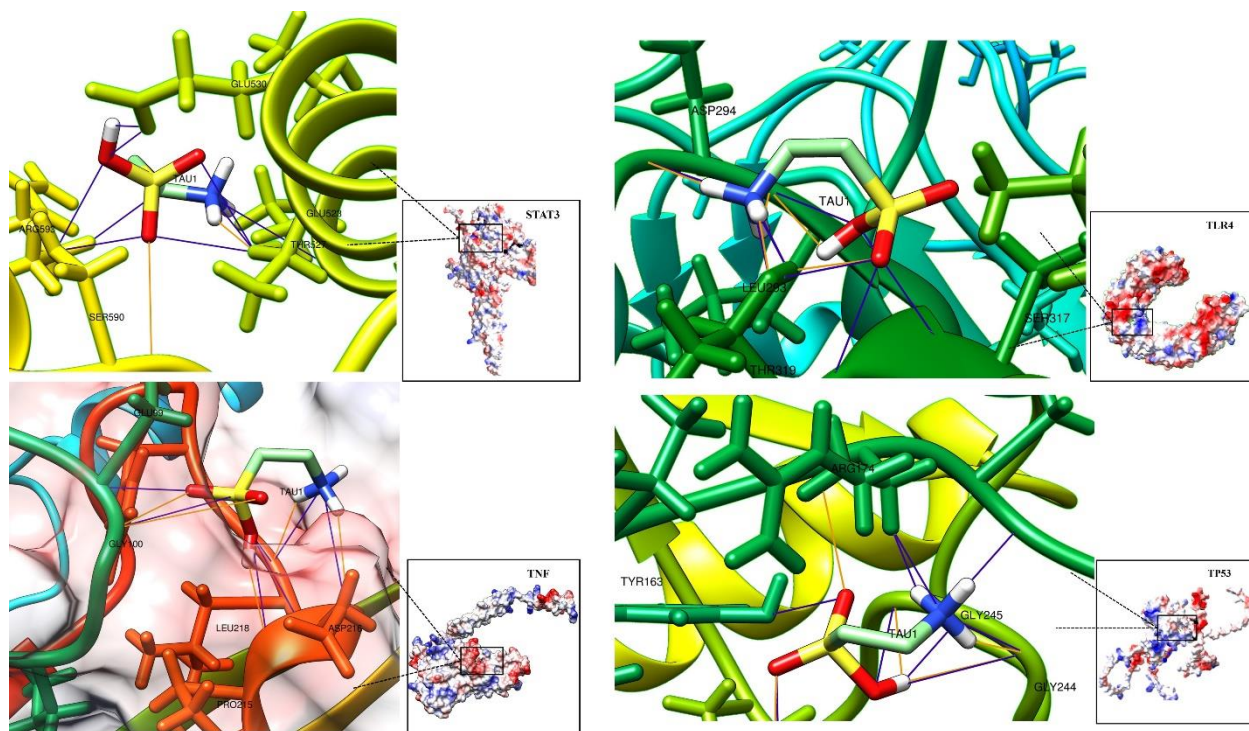


Figure 24: Taurine binding to STAT3, TLR4, TNF α and TP53. Orange line: Hydrogen bond; Blue line: Other pseudobonds.

STAT3

Molecular docking indicated that taurine binds to STAT3 (Signal Transducer and Activator of Transcription 3) via two hydrogen bonds, and strong electrostatic interactions ([Figure 24](#)). Hydrogen bonds occur between taurine and amino acids THR 527, and SER 590. The docking score is -3.8.

TLR4

Molecular docking indicated that taurine binds to Toll-Like Receptor 4 (TLR4) via five hydrogen bonds, and strong electrostatic interactions ([Figure 24](#)). Hydrogen bonds occur between taurine and amino acids THR 319 (2 H-bonds), LEU 293, and ASP 294. The docking score at the binding site is -4.2.

TNF

Molecular docking indicated that taurine binds to Tumor Necrosis Factor (TNF) via five hydrogen bonds, and strong electrostatic interactions ([Figure 24](#)). Hydrogen bond occurs between taurine

and amino acid LEU 218, ASP 216, GLY 100 (2 H-bonds), and PRO 215. The docking score is -3.5.

TP53

Molecular docking indicated that taurine binds to TP53 (p53) via five hydrogen bonds, and strong electrostatic interactions ([Figure 24](#)). Hydrogen bonds occur between taurine and amino acids GLY 244 (2 H-bonds), GLY 245, ARG 174, and ARG 249. The docking score is -4.2.

In the [Table 9](#), the docking scores of the core targets are presented.

Table 9: Basic information on the molecular docking of taurine and target proteins.

UNIPROT ID	Molecule	PROTEIN	LIGAND	DOCKING SCORE*
P00533	AF-P00533	EGFR	Taurine	-4.7
P35354	AF-P35354	PTGS2	Taurine	-4.5
L7RXH5	AF-L7RXH5	MAPK3	Taurine	-4.3
P04637	AF-P04637	TP53	Taurine	-4.2
O00206	3FXI	TLR4	Taurine	-4.2
P31749	AF-P31749	AKT1	Taurine	-3.9
P07900	7KW7 A	HSP90AA1	Taurine	-3.9
P12931	AF-P12931	SRC	Taurine	-3.9
P01584	AF-P01584	IL1B	Taurine	-3.9
P05231	AF-P05231	IL-6	Taurine	-3.9
P40763	AF-P12931	STAT3	Taurine	-3.8
P42574	AF-P42574	CASP3	Taurine	-3.7
P10415	AF-P10415	BCL2	Taurine	-3.7
P01308	1FUB	INS	Taurine	-3.7
P01375	AF-P01375	TNF	Taurine	-3.5
P19838	AF-P19838	NFKB1	Taurine	-3.5
P25963	AF-P25963	NFKBIA	Taurine	-3.4
P01579	AF-P01579	IFNG	Taurine	-3.3
P01100	AF-P01100	c-FOS	Taurine	-3.0
P05412	AF-P05412	JUN	Taurine	-2.6

* Predictive approximation of ΔG (free energy of binding; kcal/mol)

3.2 *In silico* Identification and Validation of Taurine Targets at Different Time Points of Rat Retinal Ischemia/Reperfusion Injury

3.2.1 Potential Therapeutic Targets of Taurine at Different Time Points of Rat Retinal Ischemia/Reperfusion Injury

A rat retinal ischemia/reperfusion injury time-dependent gene profiling was recovered from the EMBL-EBI Gene Expression Atlas and a Venn diagram was plotted to identify the potential therapeutic targets of taurine at different time points (24h and 7 days after retinal ischemia/reperfusion injury; [Figure 25](#)).

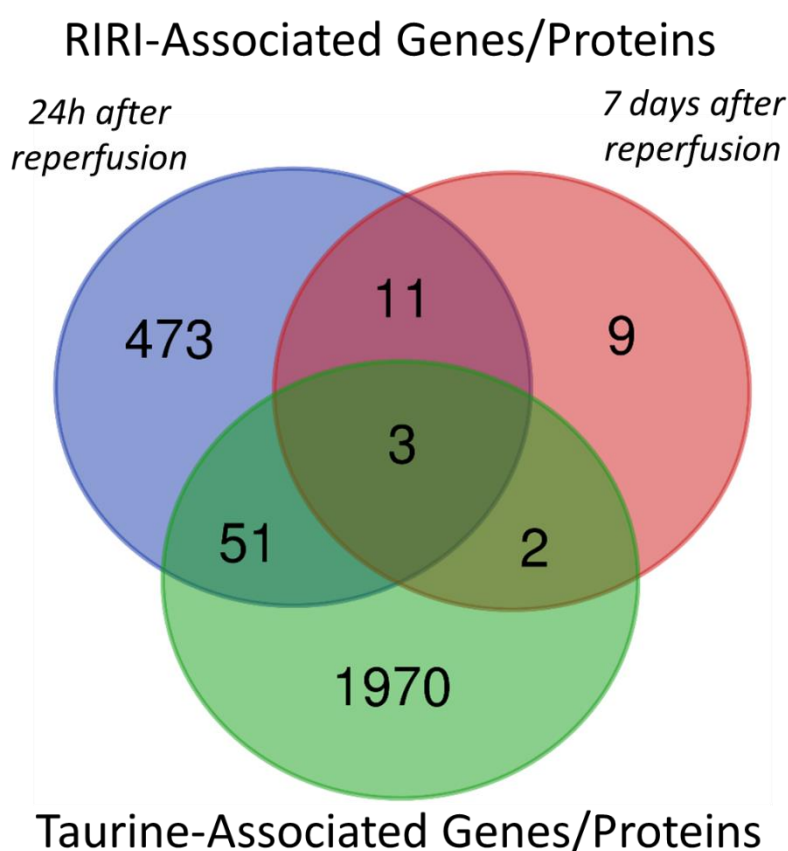


Figure 25: Intersection of Taurine-Related Genes/Proteins and rat retinal ischemia/reperfusion injury gene profile at two different time points. The Venn diagram shows the overlap between taurine-related genes/proteins and those associated with rat retinal ischemia/reperfusion injury 24h and 7 days after reperfusion, pinpointing the common finds between the two time points and between the two time-points taurine.

The targets of taurine differentially expressed 24h and/or 7 days after rat retinal ischemia/reperfusion injury induction are presented in [Tables 10-12](#).

Table 10: Common targets between taurine and 24h rat retinal ischemia/reperfusion injury gene profile. Downregulated (*red*) and upregulated (*blue*) gene expression.

ADRB1	CXCL13	HMOX1	PADI2	SLC6A6
AGT	DNMBP	HSP90AB1	PPARGC1A	SPON1
ALPL	DRD4	HTT	PRKCA	TAX1BP3
APH1A	ENPP2	IGFBP3	PSMB9	THRB
APOE	EPC1	IMPDH1	PTPN11	TIMP1
ARL6IP5	FCGR3A	JUN	QSOX1	TNFRSF12A
AXL	FLNA	KAT6A	RBBP6	USP7
BAK1	FN1	KDM4A	RLBP1	VCAM1
BRD1	GLUL	LGALS3	SEPTIN7	XIAP
COL2A1	GSTA1	MGLL	SLC29A1	YWHAB
CTSL	GUCY1A1	MMP14	SLC6A13	

Table 11: Common targets between taurine and 7 days of rat retinal ischemia/reperfusion injury gene profile Downregulated (*red*) and upregulated (*blue*) gene expression

ADGRE1	ICAM1	SLC29A1
CTSL	PSMB9	

Table 12: Common targets between taurine, 24h, and 7 days rat retinal ischemia/reperfusion injury gene profile Downregulated (*red*) and upregulated (*blue*) gene expression

CTSL	PSMB9	SLC29A1
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3.2.2 Protein-Protein Interaction Networks of Taurine at Different Time Points of Rat Retinal Ischemia/Reperfusion Injury

The common targets of taurine and 24h after Rat RIRI were uploaded to the STRING webtool to construct a Protein-Protein Interaction (PPI) networks (Figure 26). These PPI networks were then imported into Cytoscape software, where the cytoHubba plugin was used to identify key hubs and significant nodes (core targets) within the biological networks. The top 15 common taurine and 1 day after Rat RIRI targets identified through the Degree, Maximum Neighborhood Component (MNC), Maximal Clique Centrality (MCC), and Closeness ranking methods (Table 13) were subsequently analyzed using the Draw Venn Diagram webtool to identify unique molecules. The analysis revealed 16 unique molecules across all four ranking methods (Table 14). The common targets of taurine and 7 days after Rat RIRI were only 5, and that prevented us from making PPI Networks for them.

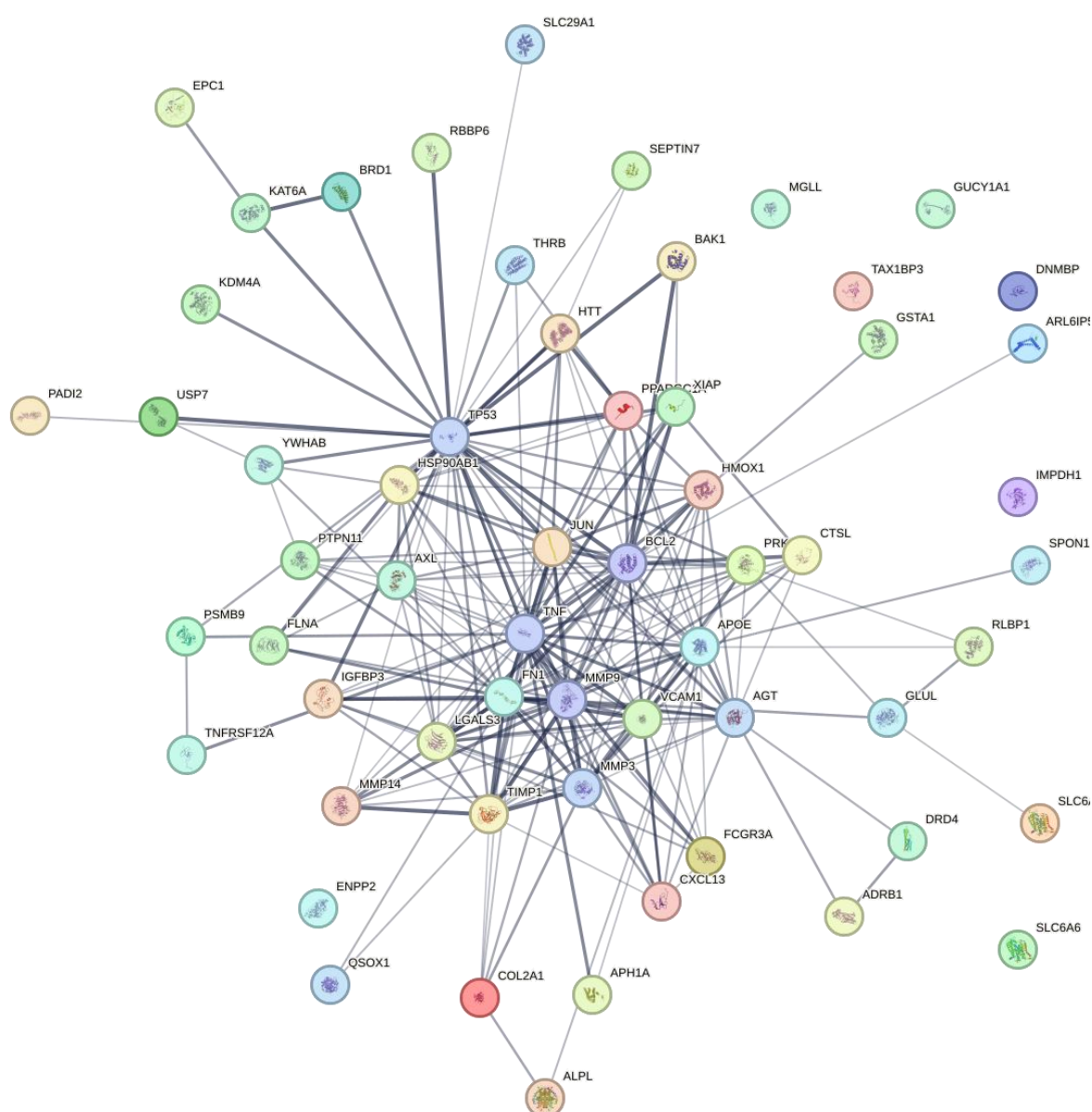


Figure 26: Common targets between taurine and 24h rat retinal ischemia/reperfusion injury gene profile [String Network](#) with minimum required interaction score: 0.4 (medium confidence)

Table 13: Top 15 taurine-rat RIRI at 24h after reperfusion targets identified through the Degree, Maximum Neighborhood Component (MNC), Maximal Clique Centrality (MCC), and Closeness ranking methods

Degree	MNC	MCC	Closeness
HMOX1	HMOX1	PRKCA	HMOX1
VCAM1	VCAM1	HMOX1	VCAM1
MMP9	MMP9	VCAM1	MMP9
AXL	AXL	MMP9	AXL
MMP3	MMP3	AXL	MMP3
TIMP1	TIMP1	MMP3	TIMP1
JUN	JUN	TIMP1	JUN
APOE	APOE	JUN	APOE
LGALS3	LGALS3	APOE	LGALS3
FN1	FN1	LGALS3	FN1
AGT	AGT	FN1	AGT
TNF	TNF	AGT	TNF
BCL2	BCL2	TNF	BCL2
TP53	TP53	BCL2	TP53
HSP90AB1	HSP90AB1	TP53	HSP90AB1

Table 14: Core taurine-rat RIRI at 24h after reperfusion targets

PRKCA	AGT	APOE	AXL	BCL2*
FN1	HMOX1	HSP90AB1 [#]	JUN*	LGALS3
MMP3	MMP9	TIMP1	TNF*	TP53*
VCAM1				

**: Molecules that are also core taurine-IRI targets*

#: HSP90AA1 is a core taurine-IRI target

3.2.3 Enrichment Analysis

The [Metascape](#) platform was used to perform KEGG and GO enrichment analyses^[145]. The taurine and 1 day after Rat RIRI overlapping targets were uploaded to the platform, with the following parameters: Min Overlap = 3, P-Value Cutoff = 0.01, and Min Enrichment = 1.5. After the enrichment analyses, the clustering network was exported and categorized according to the P-value. The top 20 enriched ontology clusters, regardless of their origine are presented in [Figure 27](#).

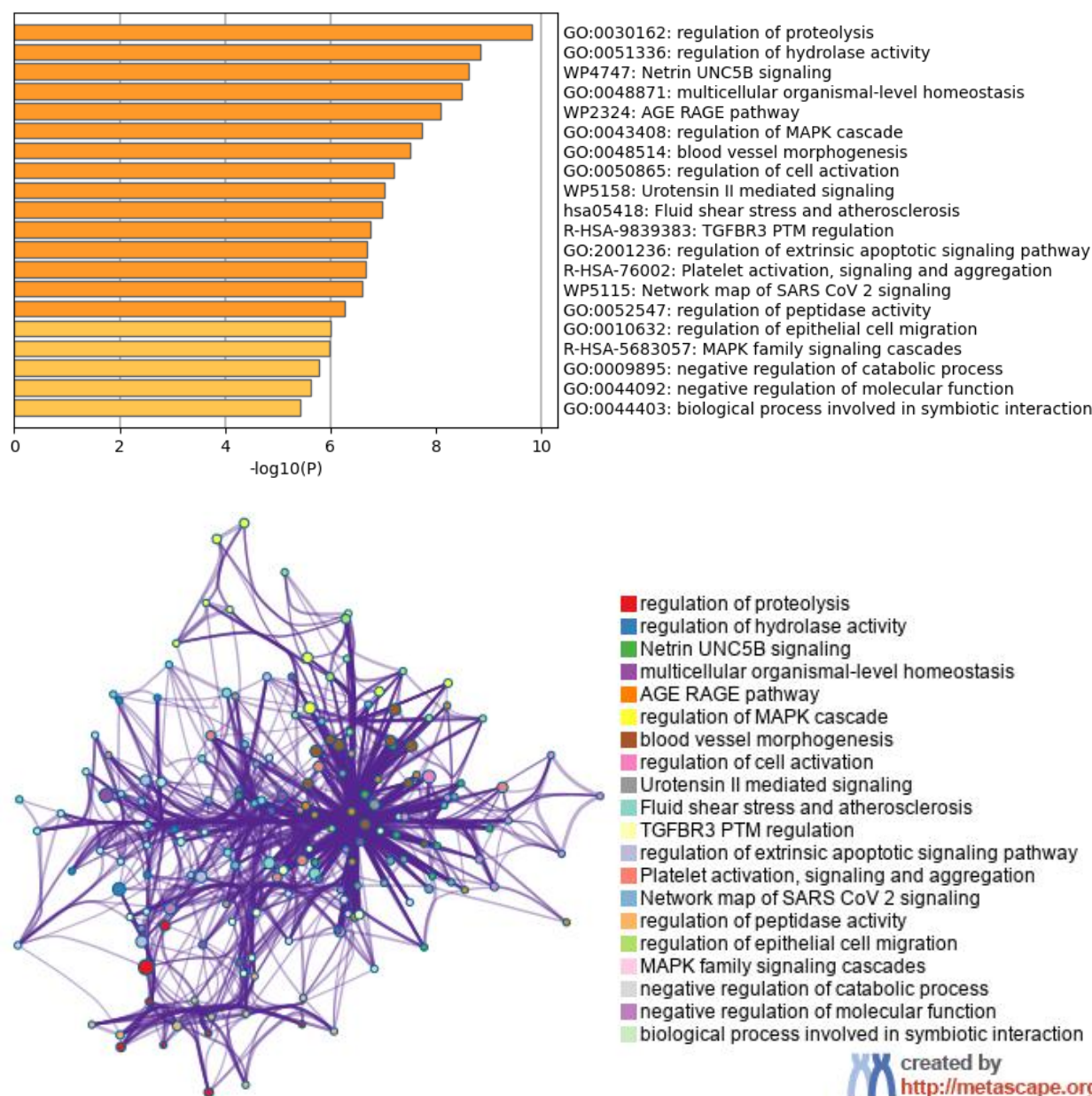


Figure 27: Top 20 enriched Ontology Clusters *Above:* Bar graph of enriched terms across taurine and 1 day after Rat RIRI overlapping targets lists by p-values. *Below:* representative terms from the full cluster converted them into a network layout. Each term is represented by a circle node, where its size is proportional to the number of input genes fall under that term, and its color represents its cluster identity (i.e., nodes of the same color belong to the same cluster). Terms with a similarity score > 0.3 are linked by an edge (the thickness of the edge represents the similarity score). One term from each cluster is selected to have its term description shown as label.

The GO enrichment analysis suggests that taurine may have early (24h) therapeutic benefits on RIRI via regulation of proteolysis, regulation of hydrolase activity, regulation of MAPK cascade, regulation of cell activation (Figure 27) by signaling pathways lipid and atherosclerosis, apoptosis, neurodegeneration, PI3K-Akt, TNF α , and IL-17 (Figure 28).

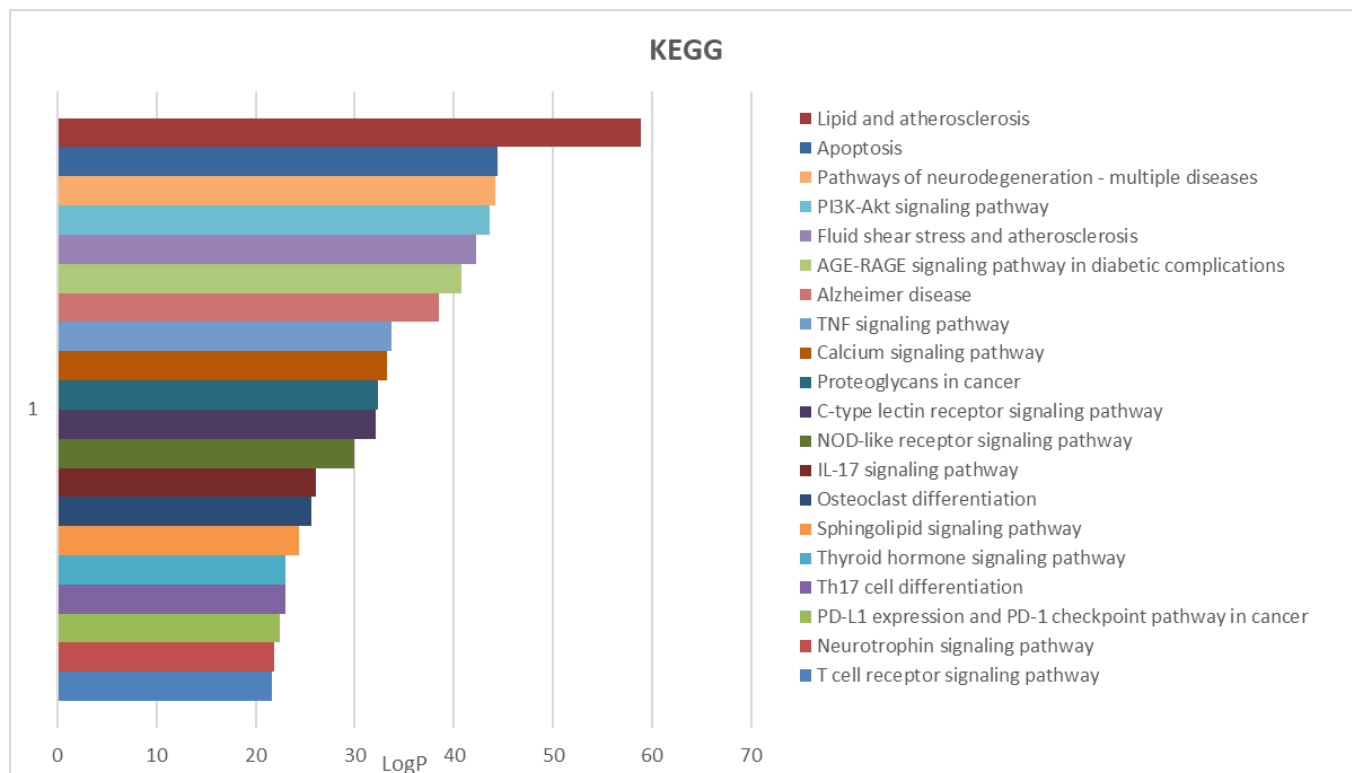


Figure 28: KEGG Enrichment Analysis. Top 20 enriched pathways (excluding those related to cancer, parasites, and infections) influenced by common taurine and 1 day after Rat RIRI targets.

3.3 Effect of Taurine on *In Vivo* Retinal Ischemia/Reperfusion Injury-Astrogliosis

Figures 29 and 30 depict the retinal astrocyte network of vehicle and taurine treated animals 24h (Figure 29) and 7 days (Figure 30) after IOP-induced RIRI in one of their eyes.

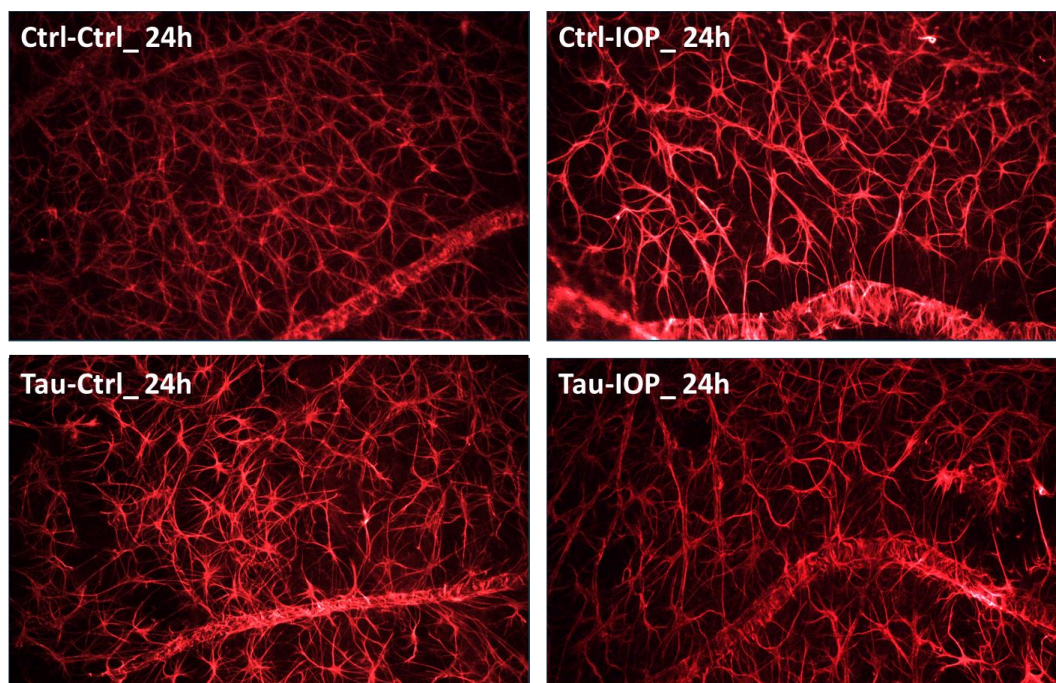


Figure 29: Effect of taurine on astrocyte network 24 hours after IOP-induced retinal ischemia/reperfusion injury. Control retina of a vehicle-treated animal (Ctrl-Ctrl), reperused retina of a vehicle-treated animal (Ctrl-IOP), control retina of a taurine-treated animal (Tau-Ctrl) and reperused retina of a taurine-treated animal (Tau-IOP) 24 hours after retina reperfusion. Scale 10X

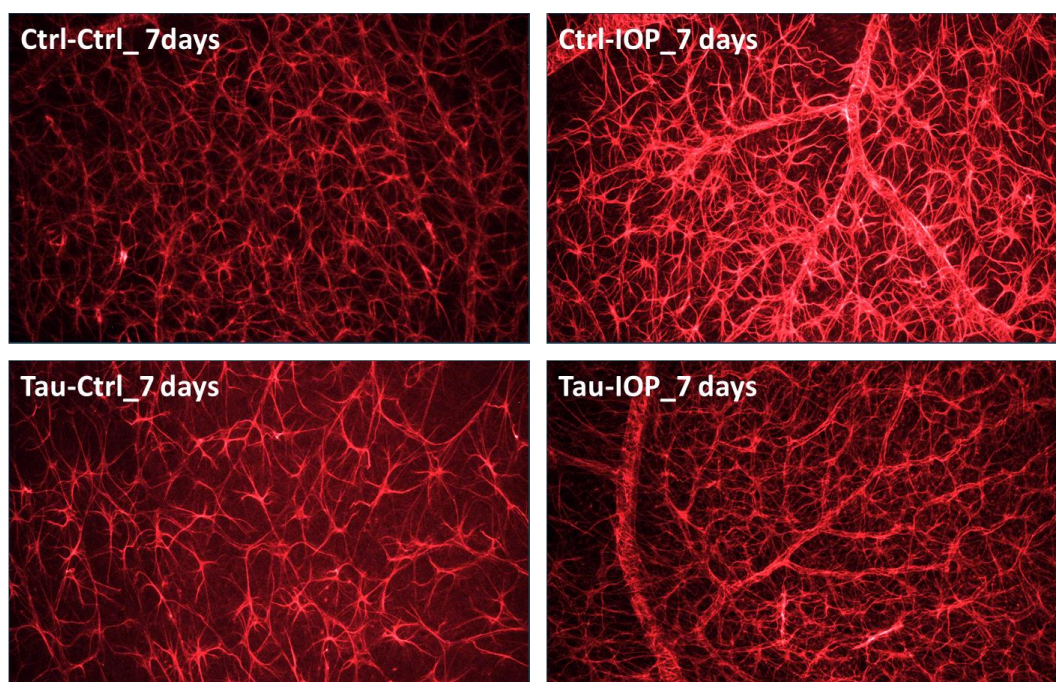


Figure 30. Effect of taurine on astrocyte network 7 days after IOP-induced retinal ischemia/reperfusion injury. Control retina of a vehicle-treated animal (Ctrl-Ctrl), reperused retina of a vehicle-treated animal (Ctrl-IOP), control retina of a taurine-treated animal (Tau-Ctrl) and reperused retina of a taurine-treated animal (Tau-IOP) 7 days after retina reperfusion. Scale 10X

3.3.1 Taurine Effect on Astrocyte Activation 24h after Reperfusion

To investigate the effect of taurine on astrocyte activation following retinal ischemia/reperfusion injury, we analyzed three morphological parameters of astrocyte activity: the number of (1) branches, (2) junctions, and (3) endpoints of astrocyte processes. As indicated by the correlation matrix, all three parameters exhibit a linear relationship ([Table 15](#)). The impact of taurine on these parameters was assessed 24 hours after IOP-induced ischemia/reperfusion injury ([Table 16](#)).

Table 15: Correlation between astrocyte activation parameters studied 24h after IOP-induced ischemia/reperfusion injury. *Correlation Matrix*

		No Branches	No Junctions	End Points
No Branches	Pearson's r	—		
	df	—		
	p-value	—		
No Junctions	Pearson's r	0.999 ***	—	
	df	56	—	
	p-value	<.001	—	
End Points	Pearson's r	0.996 ***	0.993 ***	—
	df	56	56	—
	p-value	<.001	<.001	—

Note. * $p < .05$, ** $p < .01$, *** $p < .001$

Table 16: Descriptive statistics of the effect of taurine on astrocyte activation parameters (*No of Branches, Junctions and Endpoints*) 24 hours after IOP-induced ischemia/reperfusion injury. *Mean, standard deviation (SD), Interquartile Range of data (IQR) from N number of astrocytes studied.*

	Treatment	N	Mean	SD	IQR
No Branches	Ctrl-Ctrl_24h	13	19.54	7.08	8.00
	Ctrl_IOP_24h	17	30.18	9.70	13.00
	Tau-Ctrl_24h	12	19.92	3.82	2.50
	Tau-IOP_24h	16	20.31	5.24	6.50
No Junctions	Ctrl-Ctrl_24h	13	9.23	3.49	4.00
	Ctrl_IOP_24h	17	14.53	4.89	7.00
	Tau-Ctrl_24h	12	9.33	1.97	1.25
	Tau-IOP_24h	16	9.56	2.71	3.25
End Points	Ctrl-Ctrl_24h	13	11.31	3.59	4.00
	Ctrl_IOP_24h	17	16.76	4.70	6.00
	Tau-Ctrl_24h	12	11.58	1.88	1.25
	Tau-IOP_24h	16	11.63	2.53	3.25

As shown in [Figure 31](#) and [Tables 17-19](#), IOP-induced ischemia/reperfusion injury led to a statistically significant increase of the number of astrocyte processes branches, junctions and end

points 24 hours after retinal reperfusion. Taurine reversed the effect of IOP while it had no effect in control tissues.

Table 17: Statistical analysis of the effect of taurine on the number of astrocyte branches 24 hours after IOP-induced rat retinal ischemia/reperfusion injury *Post Hoc Comparisons - Treatment*

Comparison							
Treatment	Treatment	Mean Difference	SE	df	t	P _{tukey}	
Ctrl-Ctrl_24h	- Ctrl_IOP_24h	-10.638	2.59	54.0	-4.100	< .001	
	- Tau-Ctrl_24h	-0.378	2.82	54.0	-0.134	0.999	
	- Tau-IOP_24h	-0.774	2.63	54.0	-0.294	0.991	
Ctrl_IOP_24h	- Tau-Ctrl_24h	10.260	2.66	54.0	3.864	0.002	
	- Tau-IOP_24h	9.864	2.45	54.0	4.021	0.001	
Tau-Ctrl_24h	- Tau-IOP_24h	-0.396	2.69	54.0	-0.147	0.999	

Note. Comparisons are based on estimated marginal means

Table 18: Statistical analysis of the effect of taurine on the number of astrocyte junctions 24 hours after IOP-induced rat retinal ischemia/reperfusion injury *Post Hoc Comparisons - Treatment*

Comparison							
Treatment	Treatment	Mean Difference	SE	df	t	P _{tukey}	
Ctrl-Ctrl_24h	- Ctrl_IOP_24h	-5.299	1.31	54.0	-4.0493	< .001	
	- Tau-Ctrl_24h	-0.103	1.42	54.0	-0.0721	1.000	
	- Tau-IOP_24h	-0.332	1.33	54.0	-0.2501	0.994	
Ctrl_IOP_24h	- Tau-Ctrl_24h	5.196	1.34	54.0	3.8803	0.002	
	- Tau-IOP_24h	4.967	1.24	54.0	4.0150	0.001	
Tau-Ctrl_24h	- Tau-IOP_24h	-0.229	1.36	54.0	-0.1690	0.998	

Note. Comparisons are based on estimated marginal means

Table 19: Statistical analysis of the effect of taurine on the number of astrocyte end points 24 hours after IOP-induced rat retinal ischemia/reperfusion injury *Post Hoc Comparisons - Treatment*

Comparison							
Treatment	Treatment	Mean Difference	SE	df	t	P _{tukey}	
Ctrl-Ctrl_24h	- Ctrl_IOP_24h	-5.4570	1.27	54.0	-4.2939	< .001	
	- Tau-Ctrl_24h	-0.2756	1.38	54.0	-0.1996	0.997	
	- Tau-IOP_24h	-0.3173	1.29	54.0	-0.2464	0.995	
Ctrl_IOP_24h	- Tau-Ctrl_24h	5.1814	1.30	54.0	3.9840	0.001	
	- Tau-IOP_24h	5.1397	1.20	54.0	4.2779	< .001	
Tau-Ctrl_24h	- Tau-IOP_24h	-0.0417	1.32	54.0	-0.0316	1.000	

Note. Comparisons are based on estimated marginal means

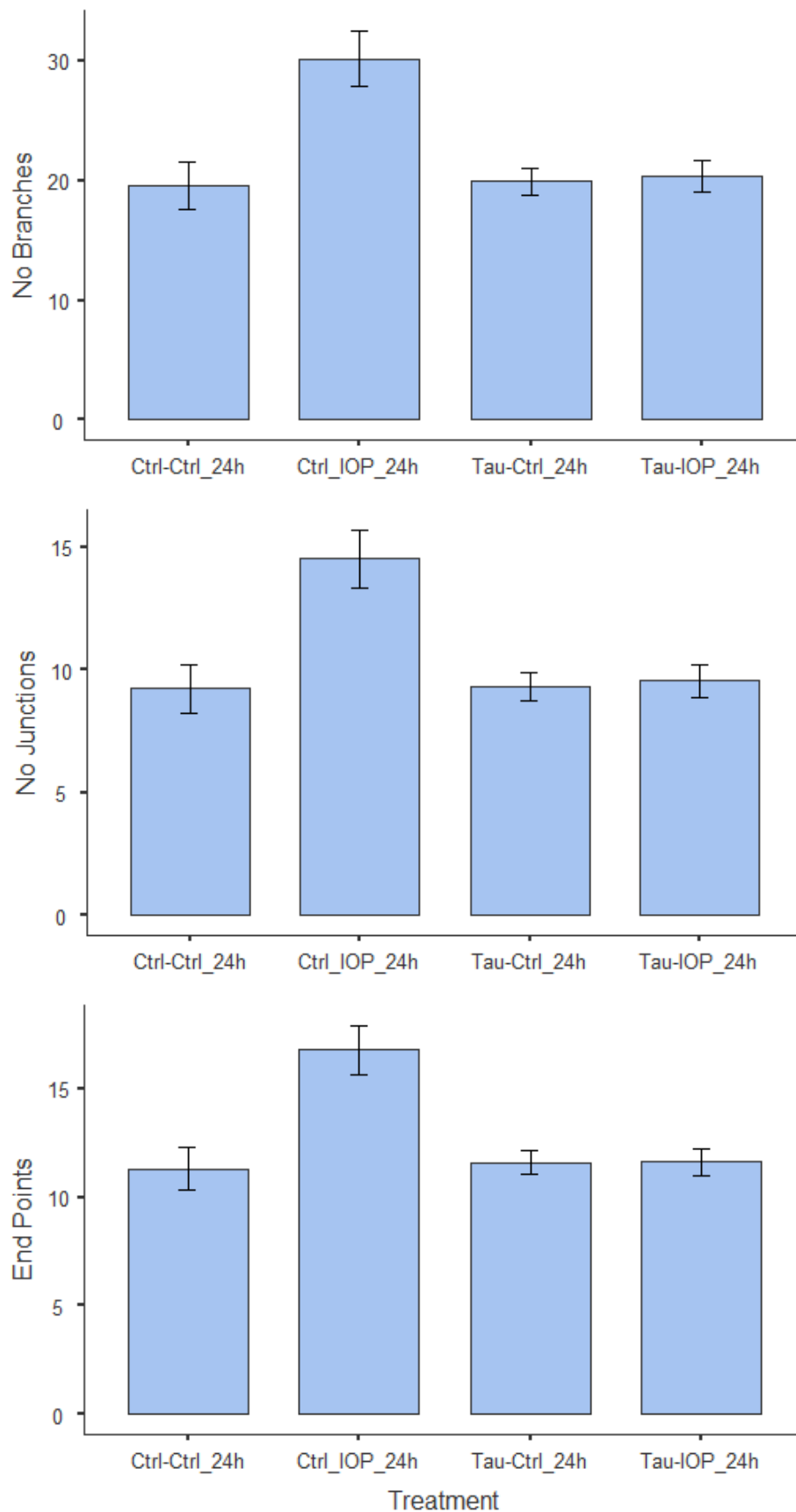


Figure 31: Effect of taurine on astrocyte activation parameters 24 hours after IOP-induced rat retinal ischemia/reperfusion injury. Control (Ctrl-Ctrl) and reperfused (Ctrl-IOP) retinas of vehicle-treated animals, Control (Tau-Ctrl) and reperfused (Tau-IOP) retinas of a taurine-treated animals 24 hours after retina reperfusion.

3.3.2 Taurine Effect on Astrocyte Activation 7 Days after Reperfusion

As mentioned above, to investigate the effect of taurine on astrocyte activation following retinal ischemia/reperfusion injury, we analyzed three morphological parameters of astrocyte activity: the number of (1) branches, (2) junctions, and (3) endpoints of astrocyte processes. As indicated by the correlation matrix, all three parameters exhibit a linear relationship ([Table 20](#)). The impact of taurine on these parameters was assessed 7 days after IOP-induced ischemia/reperfusion injury ([Table 21](#)).

Table 20: Correlation between astrocyte activation parameters studied 7 days after IOP-induced ischemia/reperfusion injury. Correlation Matrix

		No Branches	No Junctions	End Points
No Branches	Pearson's r	—		
	df	—		
	p-value	—		
No Junctions	Pearson's r	0.998 ***	—	
	df	33	—	
	p-value	< .001	—	
End Points	Pearson's r	0.997 ***	0.993 ***	—
	df	33	33	—
	p-value	< .001	< .001	—

Note. * $p < .05$, ** $p < .01$, *** $p < .001$

Table 21: Descriptive statistics of the effect of taurine on astrocyte activation parameters (No of Branches, Junctions and Endpoints) 7 days after IOP-induced ischemia/reperfusion injury. Mean, standard deviation (SD), Interquartile Range of data (IQR) from N number of astrocytes studied.

Descriptives					
	Treatment	N	Mean	SD	IQR
No Branches	Ctrl-Ctrl_7D	4	23.00	4.24	6.00
	Ctrl_IOP_7D	8	40.00	13.60	14.25
	Tau-Ctrl_7D	14	19.14	5.27	5.25
	Tau-IOP_7D	9	21.89	3.72	5.00
No Junctions	Ctrl-Ctrl_7D	4	10.75	2.06	3.25
	Ctrl_IOP_7D	8	19.38	6.86	7.50
	Tau-Ctrl_7D	14	8.93	2.67	3.00
	Tau-IOP_7D	9	10.33	1.94	2.00
End Points	Ctrl-Ctrl_7D	4	13.25	2.22	2.75
	Ctrl_IOP_7D	8	21.50	6.50	6.75
	Tau-Ctrl_7D	14	11.14	2.63	1.75
	Tau-IOP_7D	9	12.56	1.81	3.00

As shown in [Figure 32](#) and [Tables 22-24](#), IOP-induced ischemia/reperfusion injury led to a statistically significant increase of the number of astrocyte processes branches, junctions and end points 7 days after retinal reperfusion. Taurine reversed the effect of IOP while it had no effect in control tissues.

Table 22: Statistical analysis of the effect of taurine on the number of astrocyte branches 7 days after IOP-induced rat retinal ischemia/reperfusion injury *Post Hoc Comparisons - Treatment*

Comparison		Mean Difference	SE	df	t	p _{Tukey}
Treatment	Treatment					
Ctrl-Ctrl_7D	- Ctrl_IOP_7D	-17.00	4.69	31.0	-3.622	0.005
	- Tau-Ctrl_7D	3.86	4.34	31.0	0.888	0.811
	- Tau-IOP_7D	1.11	4.61	31.0	0.241	0.995
Ctrl_IOP_7D	- Tau-Ctrl_7D	20.86	3.40	31.0	6.141	< .001
	- Tau-IOP_7D	18.11	3.72	31.0	4.864	< .001
Tau-Ctrl_7D	- Tau-IOP_7D	-2.75	3.27	31.0	-0.839	0.836

Note. Comparisons are based on estimated marginal means

Table 23: Statistical analysis of the effect of taurine on the number of astrocyte junctions 7 days after IOP-induced rat retinal ischemia/reperfusion injury *Post Hoc Comparisons - Treatment*

Comparison		Mean Difference	SE	df	t	p _{Tukey}
Treatment	Treatment					
Ctrl-Ctrl_7D	- Ctrl_IOP_7D	-8.625	2.37	31.0	-3.634	0.005
	- Tau-Ctrl_7D	1.821	2.20	31.0	0.829	0.840
	- Tau-IOP_7D	0.417	2.33	31.0	0.179	0.998
Ctrl_IOP_7D	- Tau-Ctrl_7D	10.446	1.72	31.0	6.082	< .001
	- Tau-IOP_7D	9.042	1.88	31.0	4.802	< .001
Tau-Ctrl_7D	- Tau-IOP_7D	-1.405	1.66	31.0	-0.848	0.831

Note. Comparisons are based on estimated marginal means

Table 24: Statistical analysis of the effect of taurine on the number of astrocyte end points 7 days after IOP-induced rat retinal ischemia/reperfusion injury *Post Hoc Comparisons - Treatment*

Comparison		Mean Difference	SE	df	t	p _{Tukey}
Treatment	Treatment					
Ctrl-Ctrl_7D	- Ctrl_IOP_7D	-8.250	2.27	31.0	-3.631	0.005
	- Tau-Ctrl_7D	2.107	2.10	31.0	1.002	0.749
	- Tau-IOP_7D	0.694	2.23	31.0	0.311	0.989
Ctrl_IOP_7D	- Tau-Ctrl_7D	10.357	1.64	31.0	6.299	< .001
	- Tau-IOP_7D	8.944	1.80	31.0	4.962	< .001
Tau-Ctrl_7D	- Tau-IOP_7D	-1.413	1.59	31.0	-0.891	0.809

Note. Comparisons are based on estimated marginal means

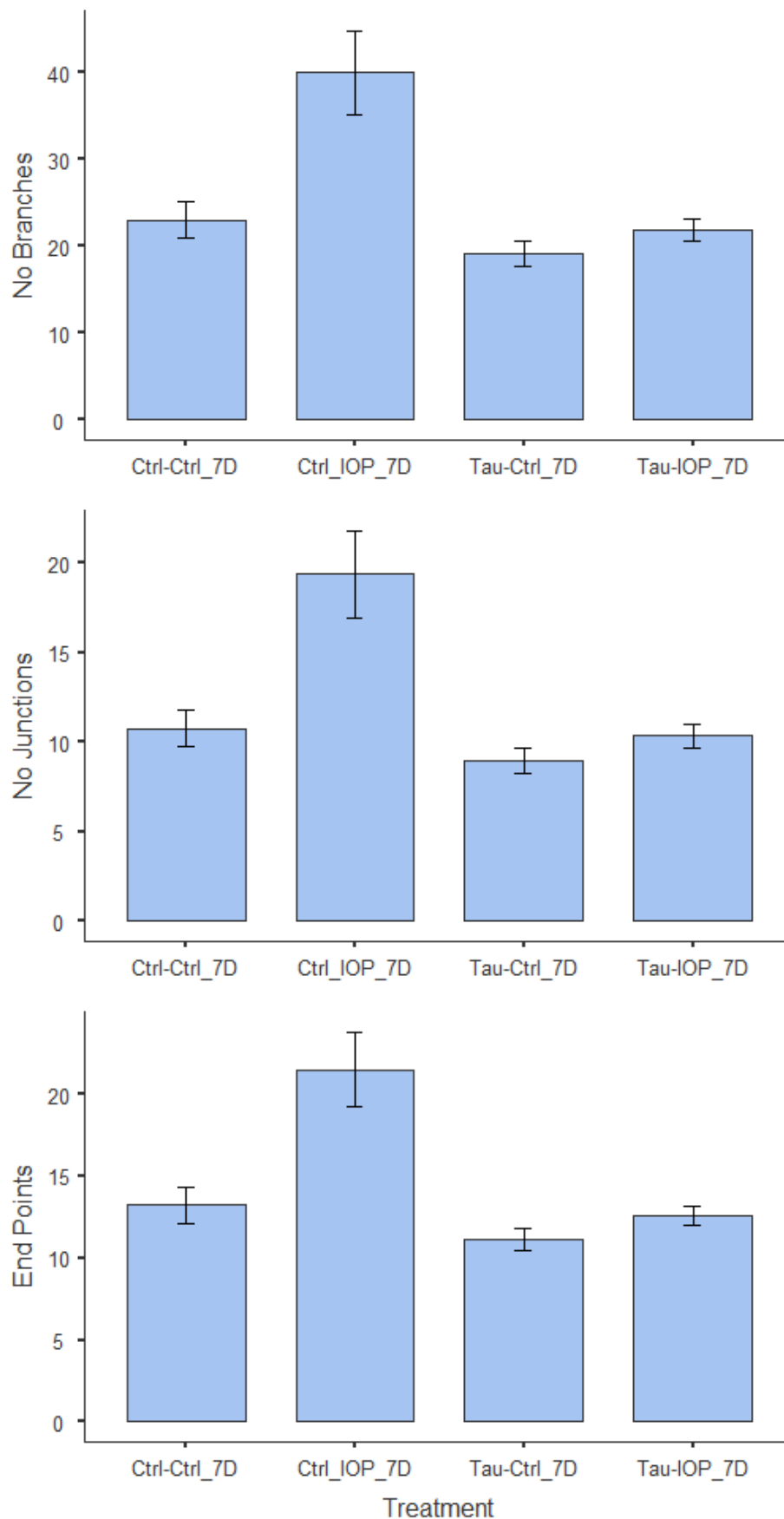


Figure 32: Effect of taurine on astrocyte activation parameters 7 days after IOP-induced rat retinal ischemia/reperfusion injury. Control (Ctrl-Ctrl) and reperfused (Ctrl-IOP) retinas of vehicle-treated animals, Control (Tau-Ctrl) and reperfused (Tau-IOP) retinas of taurine-treated animals 24 hours after retina reperfusion.

4. DISCUSSION

Taurine is a naturally occurring sulfur containing semi-essential amino acid present in the body^[11]. It is the most abundant free amino acid in the nervous system and muscle tissue and is particularly concentrated in the retina. The retina is the tissue with the highest metabolic demand in the body, making it highly vulnerable to ischemic damage^[96]. Ischemia/reperfusion injury (IRI) refers to the paradoxical phenomenon where cellular dysfunction and tissue death increase after blood flow is reestablished in previously ischemic tissues. This highlights the complexity of the damage caused by both ischemia and reperfusion^[94]. The aim of the study was dual; to create a robust framework for studying the pharmacological effects of taurine on retinal ischemia/reperfusion injury (RIRI), and to develop a versatile *in silico* model widely applicable in drug-target evaluation for complex multifactorial diseases. The *in silico* part, included network pharmacology and molecular docking studies for the identification of taurine targets in ischemia/reperfusion in general and retinal ischemia/reperfusion, in particular. Following the *in silico* analysis that identified taurine targets, astrocyte activation was evaluated *in vivo* using immunohistochemistry to confirm the effect of taurine on the tissue. The phenotypic activation of retinal astrocytes was assessed using the astrocyte marker glial fibrillary acidic protein (GFAP), which is widely recognized as a reliable indicator of astrocyte activation^[61].

Among the 2,097 taurine-related genes/proteins and the 1,991 IRI-related genes/proteins obtained from 9 databases and webtools, and 3 disease-specific databases respectively, 419 common taurine-IRI targets were identified. **GO** and **KEGG enrichment** analysis of these molecules revealed that the common taurine-IRI targets are mainly located in extracellular vesicles, clathrin-coated endocytic vesicles, synapses, inclusion bodies, and peroxisomal matrix and are primarily related to (i) biological processes such as stimulus response, metabolism, homeostasis, and immune response, (ii) molecular functions such as scaffold protein binding, active transmembrane transporter activity, protein self-association, PKC binding and hydrolase activity (iii) by signaling pathways such as lipid and atherosclerosis, apoptosis, neurodegeneration, PI3K-Akt, TNF α , Ca⁺⁺ and IL-17.

The association between the [*lipid metabolism and atherosclerosis*](#) KEGG pathway and taurine-IRI common targets is the strongest found (see [Figure 19](#)). It is known that lipids and their derivatives play a role in both cellular damage and repair processes during both the ischemic and reperfusion phases of the injury^[153]. During ischemia, the lack of oxygen and nutrients disrupts energy production, leading to the accumulation of free fatty acids and breakdown of membrane

lipids. This destabilizes cell membranes, making them more susceptible to damage when blood flow is restored. Upon reperfusion, the sudden influx of oxygen leads to the production of ROS that target lipids in cell membranes, leading to lipid peroxidation, further damaging neurons, glial cells, and the blood-brain(retinal) barrier. Lipid peroxidation products, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), are toxic and can exacerbate CNS injury. On the other hand, it is known that taurine enhances the activity of intracellular antioxidants, such as glutathione and catalase increasing tissue antioxidant capacity which can lead to a reduction in the production of reactive oxygen species (ROS)^[22] minimizing lipid peroxidation. Lipid metabolism generates pro-inflammatory molecules like eicosanoids (e.g., prostaglandins and leukotrienes) through the activation of phospholipase A2. These molecules amplify inflammation and recruit immune cells, worsening tissue damage during CNS IRI. Pro-inflammatory cytokines (TNF- α and IL-1), secretory phospholipase A2 IIA, and lipoprotein PLA2 are associated with vascular inflammation. TNF- α and IL-1 modify lipid metabolism and promote the synthesis of eicosanoids, ceramides, and ROS, which increase CNS injuries and affect several neurological diseases^[154]. Sphingolipids, particularly ceramides, are involved in signaling pathways that regulate cell death and stress responses. Elevated ceramide levels during ischemia/reperfusion can trigger neuronal apoptosis, contributing to further to CNS damage^[155]. On the other hand, certain lipids, such as omega-3 fatty acids and their metabolites, have protective roles by reducing inflammation and oxidative stress^[156]. They may help in repairing cell membranes and reducing neuronal damage during reperfusion. According to [Kegg.jp](#), the “lipid metabolism and atherosclerosis” KEGG pathway is interconnected with several other KEGG pathways highlighted in our taurine-IRI target enrichment analysis, such as apoptosis and the Ca⁺⁺, p53, PI3K-Akt, and TNF signaling pathways. The association between [Apoptosis](#) and [neurodegeneration](#) KEGG pathways and taurine-IRI common targets is also high. Retinal ischemia/reperfusion injury is associated with RGCs apoptosis with these cells being the most sensitive to RIRI retinal cell type subjected to neurodegeneration^{[96],[98]}. Photoreceptors are also prone to apoptotic cell death after RIRI and exhibit higher caspase-3 activation compared to RGCs^[157]. Taurine can prevent mitochondria-mediated apoptosis by inhibiting caspase-activation, restoring the Bax/Bcl-2 ratio or blocking Bax translocation to the mitochondria^[22].

Additionally, the KEGG enrichment results for [calcium signaling](#) agrees with the current research because it is generally recognized that Ca²⁺ serves as an important mediator of neuronal cell death during ischemia^[97]. Moreover, taurine acts as a protective agent against excessive calcium accumulation and safeguards against a decrease in energy generation^[22].

The association between the [PI3K-Akt](#) KEGG pathway and taurine-IRI common targets is also strong. It is known that the serine/threonine kinase **AKT** (*protein kinase B*) is crucial in modulating several cellular activities, including metabolism, growth, and survival. In the early stages of diabetic retinopathy, high blood sugar caused by diabetes directly blocks the AKT signaling leading to retinal cell death^[158]. Another study has shown that ligustrazine is protective in experimental glaucoma by inhibiting autophagy via the activation of the PI3K-Akt/mTOR pathway^[159]. Furthermore, trimetazidine protects against cardiac ischemia/reperfusion damage by preventing excessive autophagy through the activation of the PI3K-Akt/mTOR pathway^[160]. While according to Takatani et al. (2004)^[161], taurine prevents ischemia-induced apoptosis in cultured neonatal rat cardiomyocytes through Akt-mediated caspase-9 inactivation. Taurine has been demonstrated to modulate the expressions and activities of **Bcl-2** and Bax, decrease the production of caspase-8/9^[161], and prevent the formation of Apaf-1/caspase-9 apoptotic bodies^{[162],[163]}. An *in vivo* study utilizing a retinal ischemia/reperfusion injury model showed that cleaved **caspase 3** levels increased after three days of reperfusion. The study indicated that P-Akt levels decreased after 3 and 7 days of reperfusion^[164]. The role of **IL-6** in PI3K/AKT signaling is noteworthy. IL-6 enhances the activation of PI3K-AKT/mTOR-GSK-3 β via the upregulation of GRPR in hippocampal neurons of autistic mice^[165]. Moreover, both *in vivo* and *in vitro* investigations of long-term exposure to an herbicide indicated potential disruption of blood-brain barrier function and brain tissue injury, caused by PI3K/AKT overactivation and increased IL-6 levels^[166].

The association between [TNF signaling](#) KEGG pathway and taurine-IRI common targets is also significant. TNF signaling is involved in several brain ischemia injury mechanisms including necroptosis^[167]. TNF is the main regulator of the immune system and is crucial in the spread of inflammation. Recent studies have identified tumor necrosis factor-alpha (TNF- α) as a potential biomarker for stroke^[168]. As mentioned, taurine can directly neutralize hypochlorous acid (HOCl) produce less toxic taurine chloramine^[22] which was found to inhibit TNF- α production macrophage *in vitro*^[169]. Furthermore, taurine can significantly reduce the severity of brain injury in traumatic brain injury (TBI) by decreasing several pro-inflammatory cytokines, including TNF α ^[23]. According to [Kegg.jp](#), TNFR1 signaling activates several genes, principally regulated by two separate pathways: the NF-kappa B pathway and the MAPK cascade, or by apoptosis and necroptosis. TNFR2 signaling stimulates the NF-kappa B pathway, including the PI3K-dependent NF-kappa B pathway and the JNK pathway, resulting in cellular survival^{[170]-[172]}.

There is also an association between the [IL-17 signaling](#) KEGG pathway and taurine-IRI common targets. It has to be noticed that DBSCAN clustering analysis of the core taurine-IRI targets using STRING designated IL-17 signaling pathway to also be the only cluster formed. IL-17A has been

demonstrated to facilitate angiogenesis in ischemic retinopathy. IL-17A effect on endoplasmic reticulum stress facilitated retinal neovascularization by modulating the TXNIP/NLRP3 signaling pathway in macrophages under hypoxic conditions^[173]. Further research indicated that IL-17A might trigger Müller cell activation and provide an anti-apoptotic effect on photoreceptor cells in an *in vitro* mouse model of oxygen-induced retinopathy^[174]. The study by Li et al., (2019)^[174] suggested a relationship between IL-17A and GFAP. The association between taurine and IL-7 is unknown, as current research is limited. However, a study showed that taurine suppresses the activation of NF- κ B and IL-17 signaling pathways in neutrophils from patients with acute pancreatitis (AP)^[158]. According to [Kegg.jp](https://www.ebi.ac.uk/KEGG/pathway/) IL-17 signaling is associated with several pathways, such as apoptosis, MAPK signaling, and NF-kappa B signaling.

Network data mining allowed us to recognize 20 core taurine-IRI targets (*see Table 6*). Molecular docking simulations revealed that taurine exhibits stronger binding affinity to EGFR, PTGS2, MAPK3, TLR4, and TP53 (*Autodock Vina docking score values* < -4.0). Docking scores below -4.0 generally indicate moderate to good binding affinity, suggesting that taurine may have significant interactions with these proteins. Taurine is a small and simple molecule. This simplicity can limit the types of interactions it can form with proteins. Taurine appears to interact with many proteins via hydrogen bonds. It has to be mentioned that, the docking algorithms predict static binding, but taurine's role in dynamic interactions doesn't appear. We suppose that taurine's moderate docking scores across different proteins suggest taurine acts as a modulator of biological systems rather than a strong inhibitor or activator.

Our results indicate that taurine has a moderate binding affinity for **epidermal growth factor receptor (EGFR)** (*Autodock Vina docking score value* -4.7). The EGFR family is a type of receptor tyrosine kinase (RTK) protein. Tyrosine phosphorylation of receptors activates several signaling pathways, including Ras/MAPK, PLC γ 1/PKC, PI3K/Akt, and STAT pathways^[176]. It has to be noticed that according to the network analysis performed MAPK3, AKT1, and STAT3 were also included in the 20 core Taurine/IRI targets which reinforces the possibility of a direct taurine-EGFR interaction. Taurine seems to form hydrogen bonds with LEU 325, ASN 328, and VAL 350. These regions are located in the extracellular domain of EGFR. The A loop of EGF hydrophobically interacts with Val350 and Phe357 on site 2 of L2, while Arg41 of EGF forms a salt bridge with Asp355 in L2^[177]. We could hypothesize that taurine may affect the binding of EGF, due to a change in stereochemistry at Val350 site. A molecular dynamics simulation is required to confirm whether taurine's binding significantly alters the EGF effects. Moreover, molecular dynamics could be informative concerning a probable synergistic effect between taurine and other molecules like TGF- α .

Several neurodegenerative disorders, including cerebral ischemia, may involve **EGFR**. EGFR is hardly observed in quiescent astrocytes within the normal adult brain; however, it reappears in reactive astrocytes following injury^[178]. *In vitro* and *in vivo* studies have shown that EGFR overexpression stimulated the JAK2/STAT3 signaling pathway and decreased neuronal apoptosis in spinal cord ischemia/reperfusion injury^[179]. As far as retina is concerned, EGFR expression has been observed in multiple retinal cell types including RGCs, photoreceptors, Retinal Pigment Epithelium (RPE), and Müller cells. The activation of the EGFR-ERK signaling pathway has been recently associated with Müller cell proliferation in response to neuronal cell death during retinal scarring and seems to contribute to reactive gliosis^[180]. The effect of taurine on EGFR receptor activation is currently unknown and deserves further investigation.

Taurine also exhibited a moderate binding affinity for **Prostaglandin-endoperoxide synthase (PTGS2; Autodock Vina docking score value -4.5)**. PTGS2, often referred to as COX-2, is a major response gene linked to the preservation of homeostasis in healthy tissues and the modulation of inflammation. Taurine seems to form hydrogen bonds with THR 602, THR 198, ASN 168, and ASN 368. It may affect the activity of PTGS2, possibly via allosteric modulation, enhancing its established anti-inflammatory benefits. It is known that taurine can enhance the expression of PTGS2 in human hepatic stellate cells HSCs (LX-2) cells *in vitro*^[181]. An *in vivo* study using a middle cerebral artery occlusion (MCAO) model also showed that decreasing PTGS2 expression could lower the NF- κ B signaling pathway. This could stop apoptosis and increase the proliferation, migration, and angiogenesis of endothelial progenitor cells (EPCs), which would protect mice with ischemic stroke^[182].

According to our results, taurine could also bind to **mitogen-activated protein kinase 3 (MAPK3; Autodock Vina docking score value -4.3)**. MAPK3 is also known as ERK-1. MAP kinases, or extracellular signal-regulated kinases (ERKs), function within a signaling cascade that modulates several cellular activities, including proliferation, differentiation, and cell cycle progression, in response to various extracellular signals. This kinase is activated by upstream kinases, leading to its translocation to the nucleus, where it phosphorylates nuclear targets ([NCBI](#)). Upregulation of MAPK3 reduces apoptosis in myocardium ischemia/reperfusion in both *in vivo* and *in vitro* experiments^[183]. Furthermore, the study by Schanbacher et al. (2022)^[184] revealed that overexpressing wild-type ERK2 in mice after transient occlusion of the middle cerebral artery (tMCAO) is harmful, leading to a significant increase in infarct volume and neurological damage due to increased BBB permeability, inflammation, and an increase in apoptotic neurons^[184]. Moreover, ERK1/2 is essential in autophagy. *In vivo* traumatic brain injury (TBI) in rats was observed to enhance autophagy via the ERK1/2/mTOR/Stat3 pathway^[185]. The role of ERK1/2 in

the retina is also interesting. Knockdowning of the Erk1 and Erk2 genes in mice's retinal pigment epithelium resulted in retinal degeneration. The ERK1/2 pathway directly regulates the visual cycle and is essential for the survival of RPE and photoreceptor cells^[186]. Additionally, an *in vivo* model including increased intraocular pressure in rats demonstrated a significant rise in ERK phosphorylation after 12 hours of reperfusion. This study also shows that the phosphorylation of ERK is involved in the beginning stages of necroptosis, leading to an increase in RIP3, a component of the necrosome^[187]. According to our results, taurine seems to form hydrogen bonds with ALA 212, and ILE 226. The hydrogen bonds formed between taurine and ALA 212, and ILE 226 in MAPK3 indicate an allosteric interaction rather than a direct inhibition or activation of kinase activity.

Taurine also binds to **Toll-like receptor 4 (TLR4; Autodock Vina docking score value -4.2)**. Toll-like receptors are pattern recognition receptors (PRR) that are crucial for the innate immune reaction. Excessive TLR4 activation disrupts immunological homeostasis by sustaining the production of pro-inflammatory cytokines and chemokines, hence facilitating the development and progression of several diseases^[188]. The interaction of taurine with THR 319, LEU 293, and ASP 294 indicates a potential allosteric impact on TLR4 activity. Taurine is known for its anti-inflammatory and antioxidant effects. The binding of taurine to these specific residues if true may influence TLR4's interaction with ligands, perhaps reducing excessive immune activation during inflammation or oxidative stress. In an *in vivo* IOP model it was shown that TLR4 contributes to RIRI as early as one hour and 24 hours after reperfusion^[189]. The results showed that TLR4 knockout mice showed a significant rise of neuronal survival at the ganglion cell layer^[189]. Moreover, Qi et al., (2014)^[190] observed an elevation of TLR4 immunoreactivity within the ganglion cell layer during 30 minutes of *in vivo* ischemia induced by closing the retinal arteries and 6, 12, and 24 hours after reperfusion^[190]. Currently, there are no data concerning a taurine-TLR4 interaction. This raises new interest in exploring this connection in the context of RIRI.

Finally, taurine seems to also interact with **tumor antigen p53 (TP53; Autodock Vina docking score value -4.2)**. TP53, is involved in regulating various cell cycle processes such as cell death, DNA damage response, and differentiation^[191]. Recent research indicates that p53 significantly affects many CNS tumors as well as conditions such as cerebral stroke and neurodegenerative diseases^[192]. In many stroke models, p53 deficiency or the use of p53 inhibitors may significantly decrease cerebral damage^[193]. Research has demonstrated that taurine induces apoptosis in human nasopharyngeal carcinoma (NPC) cells *in vitro*, evidenced by an increase in cleaved caspase-3 and the overexpression of p53^[194]. However, the effect of taurine on TP53 in retinal ischemia/reperfusion injury (RIRI) remains to be explored.

Our data suggest that the potential targets of taurine revealed by the molecular docking studies are crucial in various pathways related to RIRI. Consequently, the next steps will involve conducting molecular dynamics (MD) simulations and confirming these targets through both *in vivo* and *in vitro* studies.

In our *in silico* identification of taurine targets at various time points during rat RIRI -specifically at 24 hours and 7 days post-reperfusion- we found a limited number of common targets, resulting in a smaller set of core targets. This reduction may be attributed to the fact that the rat RIRI targets were derived from a gene profiling study that focus on time-dependent changes in mRNA levels associated with rat retinal ischemia/reperfusion injury^[142]. This kind of studies typically highlight the upregulation or downregulation of specific molecular targets over time, which can lead to a narrower selection of targets that reflect only the dynamic expression patterns of genes during the injury and recovery phases. Consequently, while we were able to identify targets relevant to taurine's effects, the emphasis on mRNA level changes may not capture the full spectrum of potential interactions and mechanisms influenced by taurine. The limited overlap in targets may suggest that many molecular pathways involved in RIRI are not adequately represented in the available data, emphasizing the need for broader approaches that incorporate other types of molecular data -such as protein expression levels, post-translational modifications, and functional outcomes- to gain a more comprehensive understanding of taurine's role in (rat) RIRI. This could ultimately enhance our ability to identify critical targets and pathways for further investigation, leading to a more nuanced understanding of taurine's therapeutic potential in this context.

We identified 56 common targets between taurine and 24-hour rat RIRI, which were then analyzed using GO and KEGG enrichment analysis. The results indicated that these common targets are mainly associated with biological processes such as the regulation of proteolysis, hydrolase activity, the MAPK cascade, cell activation through lipid signaling pathways and atherosclerosis, apoptosis, neurodegeneration, as well as the PI3K-Akt, TNF α , and IL-17 pathways. It is important to note that these pathways are critical not only in the taurine-RIRI target PPI network 24 hours after reperfusion but also in the taurine-IRI target network. Notably, the PI3K/Akt and IL-17 pathways involve 13 and 11 of the core taurine-IRI targets, respectively (see: [Table 6](#), [Figure 14](#), [Appendix III](#)) indicating that the neuroprotective effects of taurine^[175] could be exerted through these pathways.

Reactive astrocytes are astrocytes undergoing morphological, molecular, and functional remodeling in response to injury, disease, or infection of the CNS^[61]. Based on the pathological state that induced their reactivation, astrocytes can be classified into two phenotypes the pro-inflammatory A1 and anti-inflammatory A2^[195]. Several signaling pathways are involved in the

activation of astrocytes after CNS ischemia, exhibiting both beneficial and detrimental effects. Neuroprotective pathways, such as purinergic, NRF2, and MEGF10/GULP1, support neuronal function and tissue remodeling. In contrast, the NF- κ B signaling pathway contributes to neuronal death and inflammation. Sirtuins, PI3K/AKT, MAPK/ERK, and JAK/STAT3 pathways can either exacerbate or alleviate neuronal damage during ischemia^[195].

Based on our *in silico* studies taurine seems to affect the molecular pathways and possible bind to molecules such as EGFR^[178] known to be involved in the activation of astrocytes (*see above and Appendix III*). So, in order to investigate the effects of taurine on astrocytic activation as a result of RIRI we studied the effect of 0.2M taurine water supplementation at two time points (*24 hours and 7 days*) post-reperfusion after IOP-induced RIRI in rats. We assessed astrocyte morphology using an antibody against Glial Fibrillary Acidic Protein (GFAP) known to increase in reactive astrocytes^[196]. Reactive astrocytes are also known to exhibit a striking increase in the number and length of GFAP-positive processes^[196] so, we choose to measure the number of astrocyte processes, junctions and end points formed as markers of reactive astrogliosis-related astrocyte morphological changes after RIRI.

According to our results, IOP-induced rat RIRI led to a statistically significant increase in the number of astrocyte processes, junctions and end points 24 hours after retinal reperfusion. Taurine reversed this increase, while it had no effect on the control tissues. Despite a further apparent increase in the number of processes in IOP-induced ischemia/reperfusion injured retinas 7 days after reperfusion, taurine continues to maintain the number of processes, junctions and end points at normal levels. In a recent study, taurine has been found to exert a neuroprotective effect by decreasing GFAP levels in the cerebellum in a rat model of LPS-induced chronic-recurrent neuroinflammation^[197]. We propose that taurine reduces astrocyte hyperactivation induced by retinal ischemia/reperfusion injury.

Nevertheless, the mechanism behind this phenomenon remains unidentified. Further studies will clarify the molecular process involved, including, molecular dynamics studies to confirm the affinity of taurine for EGFR and to assess any synergistic effects with other molecules (e.g., TGF- α), as well as to further investigate the potential interaction of taurine with other central taurine-IRI targets. Additionally, *in vitro* saturation or competition binding assays will explore the binding dynamics and assess the K_d of taurine for the targets identified *in silico* while, *in vivo* and *in vitro* studies will confirm the effects of taurine on the expression and activation or inhibition of core taurine-IRI targets during the various phases of RIRI.

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6. APPENDICES

6.1 Appendix I: Taurine-Associated Gene

RUNXBP2 VEGFR2 DCCK1 TAH1 CDN1 BBOX1 CYP26A1 PDF CYP11A1 PRR1 HSD17B1 GLUR2 KLHL2 H3-3A HSD17B10 B1AR DDB1 CDT2 UNQ249/PRO286 MITF NTSR1 HSPCB GYS1 DCP HAUSP SGLT1 UBB TCRBC2 NKG5 CLG4B CYP2A3 STK16 L16 CHRM5 FGFR1 HINT HLA-DRB5 AD1 PIR TROB1 PRO1608 NDP52 DUS2 P2PR CDC39 CPP32 IGFR3 NSPC1 NGAL HEXC JWA LHB CD16B IGKV3-20 IGHG4 GAT1 IMPDH2 FOR TLR1 GRP58 SMC3 NR3C3 PDIA3 GABATR UNQ584/PRO1154 MNK1 G22P2 CECR2 TLA519 JM2 DPDE2 ASABL SSTR2 FOXP3 BIG3 CES2 BOG MLK2 MKNK1 PPIL1 IRTKS UNQ2520/PRO6013 FACVL3 IL1RRP BHMT JHDM3A ERK1 IDO AIRE GMPR ALG2 PIN1 CD28LG MGLUR2 HSPC320 SDR3 SHBG IGHG2 PREP FCGR3 IGH C8G MST GPRK7 EIF1AX OTASE MYO15B LRRC4C DRD3 SLC6A6 ADRA2R PI12 LAD PDZRN3 GPD1 EIF1A NCC3 HEL-S-70 HLA-DRA CGI-98 THRB ADRA2A DHFR2 ALAD F7 CNOT1 PIG19 SRP72 RTA CRMP2 UNQ579/PRO1141 PRKM3 ESRRA LDHB NMOR1 HTR1DB MIP1 LTBP1 NDUFA2 FAK1 KDM5A UNQ354/PRO618 H3F3B UFD2 PRDX4 CGA SERPINA8 KDM4B HSD48 CHRNB2 TSF1 ATP5PD UNQ153/PRO179 EPOX MLN64 C1orf116 NQO2 TOB CEL FLK1 TACE SPRY2 GLP MIP5 GABRQ ALKBH PBEF1 TRIM19 ADORA2 SK2 BCMA AURKA NLGN4 ADRA1A PTPN2 API4 NIFL KISS1R CDC25L AAMDC NOS2A JBP1 CCDC109A DHFR PADI5 IL1BC RIP14 PPD ERBB DEFA2 MAST205 KLKB1 RBBP6 HDAC3 UNQ502/PRO474 RAPT1 NK1R HDCMD47P PTP2C SAHH GABBR1 BCA1 CHRM4 SCN3A PABPC2 KIAA0525 TXAS EFHA1 SEC31L1 AML1 SSRP1 SEPTIN7 RBM9 KIAA0099 LTA4 EIF4E2 ADPRTL2 NGL C1orf7 SKB1 FTH1 HO1 CXXC3 OMD HCA137 WARS1 PFC GRIP1 NFRKB KIAA1366 PGFS HIST2H3D CD32 CMA1 FCG2 LXN PCAR1 ADRB3R PC3 CREBBP CCL14 CGI-104 DUTT1 ABCC2 UNQ334/PRO533 HRNBP2 UNQ690/PRO1335 VSGP DCT DEFA1B XRCC4 RABGEF1 TNFRSF12 CGI-78 MYST3 FNTB Slco1a1 CR_09180W_A XDHA DERP11 TYRP1 TACR2 NMOR2 TNFRSF17 PPOX LGR5 HRMT1L3 RAB5A EPHX PABP BCL2L4 HSPC146 THR1 KPNB2 CDMP1 CDC45L2 PHGDH NR3A1 EPS15 My038 NHP2L1 CDC10 FDFT1 C1orf12 ZNF265 SQLE NR2B1 GABRE THRM SAK BRUNOL4 CNOT7 ZNFN1A1 SERT ZCCHC1 AFX1 GAD2 RENOX RUNX1T1 CTSK MCNAA CDW1 ADCP2 NET1 ARK1 PRA2 SCYA15 GABRG3 RAFT1 NACA XE169 NUDT22 NMDAR1 GPX4 WIF1 PSF PARC PDI TGFB CPA4 CMKBR2 DPDE4 GRB1 HMBOX1 GPRC1H CALCOCO2 CNTN5 ITGB8 DEFA5 RACH1 HSPC172 FTP3 JMJD5 NCL1 CPB1 RING12 HXB TL1 XAP1 ESTRB ENPP2 CDS2 INO80G CDR SCYA18 NPYY1 BHLHE75 PSEN2 ADRA1D SPG33 WWOX BIM MAVS CO4 YES1 BKR2 polmu SIR2L5 HO PANK3 FARS2 SIR2L6 GRMP CA12 HLA-DQA1 TAN1 KIAA0119 PTD015 SGLT2 JMJD2A MLN19 MMP12 TACI TK2 TNFRSF25 GABRA1 UBE2T CHES1 CD28LG1 BAK GPRC2A PNAS-137 GPRC1C RHO MSTP135 TP53I3 NHE1 RPS6KA2 IMPD1 STK15 ERG1 GPR67 MOR1 TDP1 TCRA CREG TAC1R KMT1D GPR49 SEPT7 GNLY FSHB KDM4A PNP1 KIAA1783 CDH3 DPRP2 EI24 ANGPT5 APJ FAK ZFP692 FIH1 NCOA5 KIAA1575 KIAA0807 CASQ1 DUS2L PDCD6 MDGA1 MC1 IGDCC2 B3AR LSD2 KDM5B AMAC1 IL1F4 NALP3 ME2 CD1B GRM3 FCGR2A H3F3A MC4R WDR61 NECTIN1 EF1G NLGN4X RAF RCOR1 MKNK2 MLK3 HSPC127 CCS JHDM3B CCL18 CYM GPRC3A ENDOGL1 BIRC4 CAM1 STK6 ERCC4 HDAC4 GLRA1 PDNP3 AD-022 FOXN3 CYH HSD17B3 ASB NHLRC2 HLAA BIRC6 HSPCA UNQ2425/PRO4984 TACC3 ADRB2R PYPAF1 TPSB1 UNQ191/PRO217 IGFR2 ICMT AD-006 AKT2 KIAA0684 IKBKB IFI53 CAF1 HLA-DP1A RP105 TNPO1 SIRT5 UNQ487/PRO1003 DPYSL1 ERBB2 GPRC1E PRPF31 NEO1 NAAA PRSS14 IBP72 H3.3A MAMDC3 HSPC150 ENDOGL2 MN KYAT1 RBP2 CYP A LRP8 TTRAP ESR TAX1BP3 CREBP1 OX MNPEP ENPP7 HLA-DPB1 ENPEP BMP2A HSD3B2 UTE PRAS40 ARL6IP5 GABRD CEK PRO1146 PSEC0052 GABRP G22P1 APOER2 CPA3 HDAC8 END SRP54

GPR35 STAR CTSO2 KLB PIK3R2 UNQ401/PRO738 SNAPC5 T6BP SCYA1 CDC45 PDE7A MBTD1
 NDUFA1 P2RU1 C2 APO3 RASGRP DPP9 NCOA1 MGLUR3 IGHG1 GMPR1 HBGFR HPTA FBP1
 NTRR DRD4 CCL1 NAGT CMKBR5 PAI1 IAK1 PKCI1 TGS1 NKA GPR40 ETRA WOX1 PUMH1
 BLYS ULIP LZM MTMR15 IGHG3 CLG4A MMIF GLUT1 METAP2 PAD4 AF1P H3C15 ASPP2
 PRKM1 KIAA0679 HEL-220 CDK2 RBFOX2 KIAA0905 CRMP1 UNQ727/PRO1409 PDE4B PGR
 PRKM2 GPR84 CASTOR1 KRT7 MC3R ADRA2L1 ZZZ2 CBFA2 NOT1 HTR1D UNQ201/PRO227
 POLE N/A WIP CYP17A1 RASGRP4 FFA2 PKIA UNQ1915/PRO4380 AXOR12 JMJD2D GABRB3
 KIAA0288 tauD CPSD FECH VESPR NOXA2 DHRS4 LAMTOR4 HLASB AAG TNFRSF13B
 JARID1A ADAP2 AML1T1 SDR25C2 RAPGEF2 AREBP DEF1 DHLAG OR51E2 UNQ374/PRO710
 TNFA BMP14 MCR KIAA1580 ATX GP3A NEU FLN FNTA BRAF1 IKKB CA5B RPS6KA1 EIF4C
 WSL KDM1B PAK4 APH1B KIAA1260 BCL2L3 GABRG2 RAFB1 HTR2 L2DTL LYZ DR3 CSD RSK1
 ITGAB VR1 NCOA2 SLC2A2 ASML3A GABRA5 OPRD GPR43 DMD NR3B1 PTP1B GHRHR 5HT3R
 PLT IMPDH1 CLGIACP6 ABCB11 NTRKR3 RRP6 XRCC5 CAM MRS; INHA ZFYVE27 TRN LMOD2
 CDABP0092 KIAA0601 CR MOP1 AGAT SH3PXD1A SCL MGLUR8 PFD H3-3B ADRAR TAC2
 TADG15 ASF1A CTSA WIPF1 AGXT POLD1 KRAS2 CANPL3 OPRK ACSVL6 GP110 FN PCPB
 KLHL11 UNQ851/PRO1800 POLR3F BLC ALAS2 CTSO HLA-DRA1 NDR1 UT1 DEK FIP3 C6orf193
 TRAV20 IDE MLR MRP MAPKAPK1A CYB5A PP3360 ULIP2 LMP2 SKIC8 PTD009 ALT2 ZRF1
 HSPC166 AYK1 COMMD9 GPRC1B GALNS TMLHE UNQ190/PRO216 LIN28A CPB HYRC1
 CLEC10A HSP75 KIAA1740 NAT1 UNQ856/PRO1865 DDH1 SCYA24 GBA1 A4 SCYD1 HINT1 NDP
 CPGL2 RNF71 CACH5 NTT HYRC BRAF GCN5 BTAK KLK3 GEMIN5 GIGYF1 CBG20456 PIM2
 GGT5 CFD PYCARD BAAT BACE ENPP3 MBD1 FTH RBBP10 G250 XIP NCOA6IP GPCR97
 P67PHOX NIF1 ALPL BBP KIAA1876 P53 P450RAI1 VISA SPHK2 ROS1 LEAP1 FNRB SLC6A17
 NCR3LG1 CNR SCYA27 RNF68 LTA4H ILC CD1C SCP3 ADGRE1 BFGFR KIAA0901 PKCA SOAT2
 DRD1 TMOD FLN1 NCC2 WSL1 BAIAP2L1 HMCES PRP31 RYR3 PNAS-118 EUHMTASE1 PIG50
 PDNP2 PMSCL DCP1 PTK1 KIAA1515 ADRA2C CCNA1 PVRL1 AIRK1 WASL FAN1 GSTA1
 BCL2L7 MAST2 RLBP1 MCH5 MTG8 CASQ CA13 DAF HDNB1 C8A CAPN3 KDM4D TIA1
 KIAA1376 CAB1 DHRS10 MLLT7 CYSLT1 GABARAP MXI2 TRIM23 CSBP2 SDF1A FLT2 DPRP1
 HSP28 ETA PO4DB CMKBR4 PLK AKR1B10 CREB2 ACR1 ABL2 LAMP1 RXRA GRM8 ALKBH1
 ARTS1 CA14 AGRT ZMYND2 WASPIP AFX NCAM LYPLA1 APNH1 LNX3 PLANH1 PHE3
 KIAA1469 CLG1 TYRO10 LNPEP KDM5C PTGDR KIAA0994 PLK1 HTR2C HTR1C PIMT C3orf8
 RNF46 RBBP9 PRKAR2A DVL2 SIR2L3 RPD3L1 MDF2 NCAM21 DRD5 SHPTP2 UNQ688/PRO1328
 ANGPTL3 ADRA2RL2 TMIGD3 CASP10 DF NPYR MPIF2 NOD9 ALR2 GCSL ULIP3 ETO TRIM31
 GLRA2 CANP3 KIAA1289 MSTP097 HER1 A-152E5.2 PNMT GRL AIK BAL ALDH4A1 KIAA0132
 SKP2 IAP4 SDF1 KIAA0677 MPHOSPH11 ACOX1 POLM HIST2H3A; BRK TNFSF15 FANCL
 ZNF692 NCF2 ART IDH2 XRCC6 DAMOX PAG1 H3C14 PASD8 RAD9A JAZ APBB1 F9 BBH ERBB1
 PICD CGRPR RASGRP2 GDIA1 SRBP AD-005 YA22 EXOSC10 PDIA1 SMCX ALDM KRAS ERAP1
 ACSB AURA MYL SNU13 HSP27 JM21 PSR GMRP STK18 JAG1 ABH LF FABPI CSBP TMPRSS6
 P5CDH CDO-1 EX33 C6orf13 IKAROS CLEC4K MID1 PLXNC1 HLA-DQB1 HSPC334 HTR3
 GABRG1 AHCY CIAS1 MTTP KIAA1271 KIAA1637 CHRN4 HUT11 FATP5 NLGN2 PENT
 AACT2 SBDN MS4A6A LSD1 BRPF2 ERR1 SRR AAG8 CCL27 CD180 FAAH CYP5A1 CDKN2
 PAD2 CPA2 KIAA1018 SRP68 KIAA1356 IL1BCE CYP2A6 MIP1A JMJD2 BMAL1 KMT4 STAC2
 KIAA1814 UNQ365/PRO701 STARD3 IGIF FCNL GABRB1 PSFL F13A GATSL3 PRKAB2 PTP1E
 LCK APPILS KIAA0762 QPCTL SBB110 ADH7 PEP ARD1 HLA-DPA1 HOT1 DGKZ SNC19 PHF9
 FOXO1A GLT1 COPS2 PCCMT ITGB6 FTHL6 SRC1 WARS ADRA1B TLR8 IAP3 PKL12 AMY2A
 MMACHC REV3L HLA-A PEPT1 TDO GABRB2 NPM2 CD207 RSPO1 GM2A PIG22 MYO15BP
 PP8675 TAC2R NKNA PRO0105 H3.3B VNRA B2AR PCK1 RAD51C SCYB13 ADO P2RY2 FRAP
 TPS2 DPYS GCN5L2 PBEF HTRL AOF2 INRF2 OPRS1 PEPCK1 SMPDL3A KDM8 CN1 APT1
 C10orf42 CYP5 NK2R SLC01A2 EREG NEMO NGN MC1R PSMB6i MAP3K10 D17S136E NAPEPLD
 GABRA2 UNQ205/PRO231 GBA FRAP1 PKR2 NR1C3 HLA-DP1B MIP4 GPR44 SAP49 CD152
 SLRR2C NXF1 GART HT004 HRMT1L5 ERP60 EPC1 DC12 QPCT KAT6A BCOR PLK4 YES NUP50

KAT2A RQCD1 HSPC275 GABRA6 PP781; CDHP ADRM1 NR1A2 MGMT IRG1 SLC6A14 TRBC2
MAC2 FFAR1 ASM TALL1 BAR MCF3 EAAT2 DDR3 RBQ1 C3orf37 UNQ455/PRO779 LIN28 MCL1
CDC45L C7orf18 ZRANB2 DOT1L G0S19-1 RCOR CTGF JARID1B BRD1 CCL24 PTGDR2 SEC31A
KDM4DL GLRA3 PTDSR LIMK2 UBA80 DRD1B ISYNA1 CLEC2C CCKRA HBRR CA7 CNOT9
PTPT FACT80 PP11246 KDM4E RABEX5 XPF SEC23A KDM1 MPSK1 RIR CALM IK1 AKR1B11
WRS UNQ3077/PRO9912 HSPC1 PTPRK TPSAB1 PAB1 TK1 TAX1BP1 FGFR MLT FARSA
PRKAR2B OGT HBXIP HPD PML CBFB SIR2L2 ADPRT2 PDE2A STARD6 SCYA5 SCYA3 ALASE
MGLL PCAF ABH1 FAP PDE5 SIR2L ZIS H3FM MNK ADRA2RL1 CYP1A2 BACE1 GPCR43
AGTRL1 CHE1 LYN CSVP FOX2 CDO1 H3C13 SLC36A1 ATAD2 C11orf67 SERPINA7 FXR ERIC1
FOXO4 NR1H1 IPS1 HTR1B CTSL1 CSBP1 RASGRP1 LERN1 GABT1 PTPL1 ADRA2B TIF2 TUBA
RCD1 SLC15A1 TPP1 ZSWIM2 UNQ292/PRO331 ENPP5 DNMBP H3F2 C7orf59 GSTK1 EAP2
SDF1B PERQ1 MGLUR5 FCGR2A1 HTT PPGB SLC6A5 ARDC3 GGT7 HINT2 TNFSF20 CREG1
TGM6 CPAMD2 HUNK1 GAT2 APH1A SEMCAP3 HBA1; ULIP1 HTR6 LAG2 CAD PPORX ZTNF4
BCLX ACAT2 HVEC SRAPD1 CCKAR COL2A1 DL1R ZNF346 ERK2 DNAJC2 AVPR1A NMDAR2A
HSP90B CSAD MGLUR7 PRO1400 PCM1 NLGN1 ALDR1 IGHV3-23 FBXL1 CRBN IGHA1 MSK8
JHDM3D LPL1 MRP1 MACROH2A1 HER2 CCBL1 SPAT ERBA2L AGRP APCS JAGL1 LDHC PC
GLYT2 hCG_43694 SFTPUB UBE4B CHD4 GABRR1 HSD17B5 SLC2A4 API3 NGL1 MC5R TRAC
LYF1 GDF5 CHIA BRL PMSCL2 ADRA2L2 CSDD1 PADI2 SDR41C1 NOXO2 KIAA1149 NAC3
CYP26 HEL-214 FCG3 ST14 DEF5 HIF1AN PRD MTS1 NR1H4 BPHL GABRA4 PDI2 CTDSPL
PRMT3 MSTP141 ARHGEF1 HIST2H3C; VTNR FNRA THUMPD1 DPP8 OK/SW-cl.84 ERP57 HRR1
DRD1L2 UNQ694/PRO1339 DCAF2 KLHL19 DAT1 PIG15 ADRB1R DIA4 KIAA0012 WDR5
BHLHE74 HDCMA22P CAP2 FKHR DTL HSP90A ABP PRAF3 NOD5 COX2 TMOD1 CACNL1A5
LPAP ABP1 EXOG ACMSD GLRB IMPD2 DKFZp686C11235 OPN2 TPS1 FLG PPP1R16B ERCC11
CD16A HLA-DRB1 TAP LY64 KIAA0319 TIP1 MT-CO2 ARHGDIA PRKAR2 TRAPPC4 MCG7
CD49D CLG FKN TBG TIMP NFKB3 MPP11 IDUA P2RX4 BAFF QSOX1 PLU1 SDR47C1 ESRL1
NPY2R HME ANPG PABPC1 MSK12 AOF1 SRC2 "ARL6IP5" BBOX JARID1C CGI-124 DRP3 BCM
HEPC B7H6 GPR54 HNL PRO2000 CRALBP cbh TNFSF2 CMKAR1 ENT1 KIAA1070 MLXIPL
APECED UBCEP1 ARHGEF36 SAPK2A AVPR1 CCL15 PDE8B SF3B4 AAT2 HSPC2 GPRC1G XYLB
CRTH2 ACOT4 BHLHE78 CBFA2T1 CRMP4 ACPL1 ZNF220 KIAA1095 TDO2 DXS1272E FE65
TRBC1 AKR1C3 PABP1 RBBP2H1 DDR2 UNQ550/PRO1107 GP2B PPP2R5E PRKCNH1 GPT2
KIAA1010 PTK6 AGT1 BCL2L JMJD2B GIG12 CPSB HNRNPH2 PYGM FLC3B UNQ1931/PRO4406
TPPA FN14 QSCN6 ENGL PRKACN1 ARFD1 MEA5 SNAP MGEA5 CHN1 JK EVR2 SCYA14
SLC14A1 RBBP2 NARC1 CBP EIF4EL3 DHODH STK38 P67EIF2 AR KIAA1007 GATM ERBA2
EDG5 MNK2 MPG INDO GABRA3 SCG5 D9S57E HDC SFTP3 PCGF1 PTPN13 IGHE PDI5 CELF4
SSTR5 TDP2 NKNAR PACT HO2 NPC1 ALDH4 RNF RAMP VEGI CES1 H3F3 VRK1 GLUC
KIAA0585 SLC27A5 LAB7 DPYSL3 LRRN6A CD26 KIAA0876 GRIA2 TOB1 CYPL1 EEF1G PTX2
LANCL1 GARS MOZ CSPB1 GGT NR3A2 IPEX SPRK UBC SES1 HBA2 PSMB9 PUM1 GLIF
HNRPH2 ROS TOP1 KIAA0071 NPAP60L FRAP2 RASK2 H2AFY

6.2 Appendix II: Ischemia/Reperfusion-Associated Gene

MSRB1 FSTL1 ACTN4 CREB3L1 CD44 MLC1 TNIP3 KCNMA1 GRAP2 NDUFS6 EDN1 PDHA1
BYSL ABCB7 COX7A2L DDT SALL1 MPL SLC25A3 CUBN IRAK1 CSPG4 IGF1 PAK1 NTRK1
CYTB FRAS1 GDF15 EP300 HIC2 PRKCG CHORDC1 TRIB3 CCNB1 HRG SLC33A1 SOD2 KCNK3
GDA AQP9 AGPAT1 MET MTNR1B GOT1 CASP6 BBC3 NR4A2 LONP1 SRPK1 SYNGAP1 NFIA
FADS1 LEP SLC22A2 SERPINF1 RPS6KA5 OXT CCL22 SHC1 SLC12A4 BRCA1 SLK TIGAR
PRKCB C5AR1 CYP2C19 A2M UTRN ROCK2 MCUB TERF2IP INTS6 MAP3K20 COL1A1 SLC8A1
OGDH HES1 GSS C1QTNF3 SRRM4 HSPA1L PPP3CA UTS2B SELL LTBP4 ATP5MC1 GPR55 MT-
ND4L CD38 TNFRSF9 CCNE1 LAIR1 YTHDF1 TIAL1 RBPJ GSTA2 FOXM1 TPI1 MAOA PF4 VAT1L
C3 GJB1 TPM3 ORM1 SLCO2A1 HSPD1 F2RL1 ULK4 FABP5 HYOU1 PMF1 PMM1 MYH6 IL1F10
SERPING1 GCLM PCDH7 MMP16 TNFRSF10A ATP6 KL SON MAFK PTP4A2 BDKRB1 RACGAP1
PIK3CB FCN1 TNXB SLC22A6 FUS DNAH11 NPR1 ABRAXAS2 NLRP5 GHRL MICU1 MFN2 ATR
GNA11 HVCN1 GRIK2 RYR1 MT3 LRRK2 LIF ACTC1 MAPK10 EPX QKI ENDOG IL5 PDP1 PTGES
MFAP5 IL6R CST3 COL7A1 CHI3L1 CLEC3B CIITA HPS4 CRP EPHA4 MUTYH HSPA8 C8orf86
IMMT PICALM SELENOP PNOC AGGF1 COMMD5 GSTP1 SLC9A3 HBG2 NOP58 STAT5A FKBP4
PAEP MT-ND2 AHNK TIAM1 PTGER1 IL18BP SMPD2 IL17C TNFSF12 PXMP2 AHR LRRC53
CHMP2B KCNA4 ATP1A1 UBE2N NFE2L2 SH3PXD2A TAB1 NRF1 HEXIM1 KLF4 YWHAZ SCN1A
JADE1 BTK OPA1 TRAF2 ECE1 BCL2L15 PARG WNT3A GOT2 ZNF667 MIR29B1 ATP7B RAB38
BMP6 PPP1R1B NPPA DCX RRAD CCL26 KCNK10 RNF182 SNAI1 NDUFA13 CYP19A1 MANF
PLA2G6 ENTPD7 SOX9 CYP3A5 BCKDK PHB2 NDRG2 PXN CAMK2B MIR29B2 TIMM17A
CADPS2 ZNF354C NGFR IL23A JPH2 C5 SLC2A10 TRIM45 IL1A PDGFD FST ERCC1 CAV3 TLK1
AQP8 ASTN2 PARK7 GSDME TAB2 SGK1 UCN2 NAIP KCTD13 TANK XKR9 RCAN1 THY1 KIT
SHH THPO EIF2S1 CREB3 TNFRSF1A SPP1 TNNT2 CASP14 GRIA1 WNT5A TRPA1 BMP7 OSM
CELIAC2 UBE2D3 OLR1 EIF5A TRIM63 MAP1A NEFL AGER CTNBN1 ENTPD2 WNT9A MICA
PPID FIS1 CYP2C9 FOXN2 NT5E FPGT-TNNI3K NPY ITGA6 BAG3 OXA1L THAP5 CCND1 TRIM35
IGFBP2 PIK3C2G NOTCH2 SLC9A2 CCL4 PDCD6IP TRPM2 ATXN2 USP18 S100B SLCO1B1 SST
MAPK7 PRF1 NFAT5 ACE2 CMKLR1 DGKQ IRS2 CSNK2A2 SIK2 IL15RA RBM3 CUL3 SCFD1
PRKACB PDE3A ZNF354A MAP2K4 ITGB2 RIMS1 PROS1 SOCS4 POR XRCC1 MAS1 LAMA1
SLC7A11 AREG TINAGL1 MME SENP3 APEX1 PAX2 GAST MYC WDR26 NTS BIRC2 FOXE3
PTGIR SOCS3 AGBL1 NLGN3 IL10RB ARID1B PCSK2 BGN GAL SLC14A2 IL13 CD86 CALM2
ICAM2 TNFAIP3 ATG3 CRLF3 FUT8 APOM PKP2 AMPD3 PIAS1 ADCYAP1R1 WWP1 FSD1 STAT1
GCLC IGFBP5 SERPINA4 RERGL IL18RAP BLVRB STMN1 HABP2 KCNQ1 EPCAM ADCY1
SMURF2 CR1 SLC22A3 BSG UCP2 ZFYVE9 AJAP1 PPP1CA PROC FPR1 PTPRC CD14 ABCC9
PPARD YBX1 ANXA2 BPIFB4 AK1 C4B VIL1 CCL11 RHOD COL4A2 HAS3 LUM PPP1R15A
CITED2 FOXP2 TRPM7 FLT1 SIRT7 SIAH2 PDIA2 ALOX12 EIF4E SMAD2 EFNA5 YWHAG ADA2
KMT2D IGF2BP2 SLC29A4 MMP3 PRKCZ TRAF6 METTL14 PLD2 BRCA2 TFAM FMR1 CHMP2A
ANGPTL4 APELA PTGER2 FOXC2 GDNF TRPC5 SCGB1A1 NTRK2 ADCYAP1 GSTM1 CRH AGRN
PRSS2 CNPY3 PLCB4 GADD45A AASS POSTN RIPK3 PLA2G1B SLC8A2 CYP27B1 FTO OCLN
NAGLU SCG2 CDON PLAT TNFSF11 YRDC BLVRA CXCL2 GNAI1 SLC25A4 MYL3 THBS4 MYLK
MAPKAPK3 SOD1 CAPN2 NR4A1 AQP3 SIRPA TRAPPC11 APOH ADAMTS8 TLR7 SMAD1
CEACAM5 POLDIP2 KCNA3 HSPA14 DBI PDGFA IL4 SELPLG SMARCA4 DIABLO MASP2
NHERF2 OPCML AKAP12 STAT5B PRPF8 NOX3 TRIM11 DYNLL1 HSPA12B PLSCR1 UBE2O
APOD TRPV4 NEK8 FBLN2 SULT1A3 PVALB OSBPL1A F2R THBS1 APOO ABCC5 COL6A5 H2AX
TCAP ABL1 PTPRG MAP2K7 TXNRD1 GZMB TMBIM6 UBE2H VIP SMPD3 CHRFAM7A NFE2L1
DAXX STIP1 HLA-G GPER1 LRP2 MYDGF SART1 MAP3K12 SLC3A2 HSPE1 CABIN1 EIF4EBP1
GCKR NTF3 APLN TRPC6 GUSB NID2 DNM2 SUZ12 PLA2G7 PDE6B TERT KRT18 SOCS1 IL17RA
ATP2B4 TRIM28 RB1 HADHB TNIP1 GLRX SAA1 FBXW7 KNG1 CLCN3 CANT1 IRF2 RUNX2 MT-
CO1 GRK4 PPP2CA IL1RN ODC1 PPRC1 MAFB GRINA TFPI THRA MAPK11 ENO3 CA3 GFER

TNNI1 ZNF143 TSPAN18 ENO1 CXCL10 GOSR2 SP3 COL4A1 VCAN SYDE1 ADAMTS3 DNAH8
 GLS SLC1A3 TGIF1 SFTPC ENTPD1 JUP MIR140 STK11 LRCH1 SAA4 PSMC5 MAP4K2 LOX
 NLRP1 LAMB2 HNF1B ACP1 CCN1 TAB3 MAP3K7 IMMP2L MARCKSL1 NCK1 CDKN1A LPL
 CPOX LAMC2 CTRL USP36 EVA1A CHADL F5 ITCH ELAVL1 GJC1 LRG1 CFLAR CDKN2C GRP
 ADM HILPDA FPR2 MLKL CD47 MAPK8IP3 LAP3 AGMO PPIC OTC RAP2C NMT1 HADHA AHSG
 TFEB FXYD1 ATG12 BMI1 FNDC3B MASP1 BLZF1 GFRA1 RBP3 NDUFB2 HAX1 ACTA1 GABBR2
 TOMM40 ADNP UBE2V1 PTPRU FGF21 POMC IL9 EIF3E SLC24A3 PSMB3 MT1A DNMT1L NPY4R
 ITGA7 TXN2 MPRIP ZFAND2A TOLLIP SORBS3 ALB C1QA CEACAM1 GRIA4 TMEM132D DCN
 NOD2 NINJ1 IL21 EPAS1 STEEP1 S100A12 PRELP TREM1 CKMT2 SPRED2 TIMP4 SLIT2 P2RY11
 CX3CR1 ANXA7 GAPDH SUCNR1 TLR2 P2RX7 MARCHF1 CASP4 KCNJ11 TPR MACROD2
 ABCC8 MTX2 SERPINA3 ADAM11 REG3A IRF9 AUTS2 MMP10 ROR1 TFF1 FSD1L HAP1 ACTG1
 GLP2R H2BC3 HAVCR2 PLAUR MAP2K3 CKM SLC4A4 KAZN ADAMTSL1 CSNK2A1 SIVA1
 RBM28 EPO APRT AOX1 PGLYRP3 LY96 RALBP1 IL1R1 CTTN PROCR SLC16A1 SLC17A5 KCNS3
 APOC1 MIR29A ACSL4 ITGAL PPM1D MLYCD NDUFA5 CYP1B1 SAT1 C1QTNF1 TRADD VEGFB
 RBMS1 HDLBP BCL2L14 MAPK8IP1 S1PR3 CHRNA7 PGR-AS1 EFS IFIT3 AVP PDE3B MEF2C
 PGK1 KLF3 ESM1 USP29 RPTOR ERBB4 UGCG DNASE1 PRCP XPNPEP2 IL12RB1 RAE1 DMRT1
 MEF2D RYK HSPB6 PANX1 RCBTB1 COX5A MYL4 AIFM1 CYBA SOS1 TGFB2 CHRDL1 RAC1
 HSPB8 LAMA2 ADD3 PTEN ANXA1 C1R AGFG1 NID1 SETD2 ATP8 ID3 S100P TICAM1 PROZ
 POU4F2 PLCB2 KLF11 TMOD4 UCN SMDT1 SLC22A4 GNAI3 NAXE ITLN1 COL20A1 TUG1 IRF1
 PTK2B UNC5B EXOC5 SDHB MBL2 SLC01B3 BIK NRP1 ALOX5 GJA1 GNAI2 PRDX6 GPNMB
 AMBP RGS16 ACVRL1 TGM2 MAP3K5 CLDN4 CSN3 TBK1 TIMP3 NR1H3 SLC44A2 ABCG1
 EPHA2 RAC2 PROK2 MTHFR SLC18A3 INTU SMAD3 ATF3 NEDD4 CACNA1E YTHDF3 APOC3
 NPPB HIF3A GGA3 KCNA2 PLA2G2A LGALS1 COL15A1 GLRX2 EZH2 PON1 HBEGF PRKCI
 CDKN1B OSMR FOXC1 SOD3 USP49 CYSLTR2 STEAP3 YEATS2 MIR223 ABCA1 EIG121 CSF1
 REST TNFSF10 TIPARP SLC8A3 DTX3L CHEK1 CRYAB RNF17 TRIM72 CKB MUC1 SLC2A3
 PTHLH MAP3K14 SLC40A1 TNFRSF11B C3AR1 CD36 TLX2 LBP CGB5 MBTPS1 SH3GLB1
 MEF2A APOL4 ADAMTS9 ABO KCNH2 TRAF3 CHD3 HMCN1 CD40 SGCB E2F1 MORN1 APOB
 CFH TSC2 CS IGFBP7 ZNF609 PRDX1 FGB CCDC83 ITGA1 FLNC SPTAN1 MIR137 ATG4B AQP4
 MERTK BAG1 CHGA HSPA4 PAPP A RHOA CALCA CXCL11 SERPINA1 TNNC1 PPIG BCL9L
 TMEM74 CRHR2 RET PRKAA1 IL1RL1 ALDH1A2 RGS19 MT-CYB CXCL8 EGR1 RIPK2 SLC12A2
 CD58 OGG1 GLP1R HDAC9 TUBA1B SELE MSX2 PSMC6 PROKR1 ABCB1 MSI1 HECTD4 CTF1
 MTF1 SLC34A1 PIK3C2A DNAJC7 NOL3 MAP2K6 HUWE1 IL17A ATP2A1 PPP1CC YY1 KCNB2
 ADGRL1 VPS51 STUB1 WT1 BNIP3 SLC22A8 STEAP4 TXNRD2 SERPINB2 AOC3 CCNL2
 MYBPC3 FABP3 TRPV2 CCN2 FAF1 CLCN2 CDK6 P2RY1 CSK CTSS COL6A2 ZC3H12A HSPB2
 CNTF MAP3K8 DLG4 ANO1 STC1 KLRK1 AKIP1 RAMP1 EGR2 LTB4R2 EFNA1 CNRIP1 ANK2
 PLA2G4A SMAD7 VLDLR VEGFC FHL2 PDGFB CALR VIM CADPS DES FGFR2 NG2 CSF3 BEST1
 PPP2CB B4GALT6 ATG5 EDNRB ASIC1 NYNRIN FEM1A NR1H2 PDPK1 CD226 CPT1B CD2AP
 CCL20 IL22 MYH10 ORAI1 IL34 AMPD1 MIR103A1 MYH11 ITPR1 DDAH2 FGF4 NPR3 SLPI
 IRAK3 BCAR1 ADAM10 TRIM27 MT-CO3 STING1 TSPAN33 TGFBR1 P2RY12 GRK2 CDC42 VWF
 VPS33B VTN CTH SEMA7A HTRA3 SLC30A4 TIE1 ATP5F1A HSPA1A DAPK2 PFKFB3 BMP4
 ADAMTS20 VSIG4 MCAM TXNL1 TRIM69 MYL1 C1SD2 SFN FOXN4 VDAC1 CASP7 TJP1 CD46
 PTPRT PTX3 PER2 DSG1 ANG SLC26A11 CCK GABPB1 SMAD4 STAT6 FXYD4 FKBP1B SLC24A2
 SCARB1 IL3 S100A6 ATG9A TFRC CAMK2N1 PTPA GREM1 PRKAG2 AGXT2 EPRS1 PEL1 ILF3
 C1QTNF9 PNPLA8 MST1 BCL2L13 SERPINF2 FBN1 HAVCR1 S100A8 TYRO3 INHBB RNF5
 LEMD3 CHKA TNFRSF13C AIF1 MAP3K1 SOAT1 ALDOA GAB2 CTNNA3 AIMP2 F8 MIEF1 EGR3
 ATF4 IL12A BMPER CCL2 TXNIP PRNP ADCY10 LAMC1 EIF2AK3 CAMK2G TLR3 HMGB2 TFG
 ANXA5 ANGPT1 CPT2 VIPR2 EEF1A1 ADPRS EIF4G1 TRPM4 LTB4R PMF1-BGLAP FXR1
 UBE2G2 RNF213 PHLPP1 ACSS2 GPR31 PSMA2 PRKCE ITGAM BLOC1S1 ROCK1 CD34 TGM1
 FOXP4 P2RY13 MGAM RTN4 COL3A1 FASLG MIR126 CACNA1D PRKAA2 CD163 PGP DPT
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 GRIK1 NUCB2 ADPRH DNMT1 NOD1 SOX5 ADAMTS1 PRKG1 INA AKAP1 NLRC5 EIF5 FABP1
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 CAV1 ALDH1A1 HPRT1 EIF2A TNFRSF1B PSMA7 GET1 CELSR1 TNNT3 THBS2 IL6ST WNT2B
 ETS1 IL22RA1 CASZ1 HSP90B1 C1D CXCL1 SORD CARTPT MYH7 TNFRSF10B SH2B3 CDK5R1
 RYR2 GRN MAP2 SLC36A2 TGFA ANPEP NPR2 PROM1 CFTR GSTM3 KCNA5 HSF1 NPPC KMT2A
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 COL6A1 STPG4 COX4I1 WNT4 IL11 F12 NUMA1 F11R KITLG SUMO4 RNF19A SPARC TXN
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 NF2 NRG1 ADAMTS13 CAST PRRT2 FBXO32 TGFB1 LEXM SESN2 CDKN3 CASP12 CLCF1 GPR39
 CYP2J2 FGD6 HSPG2 TNF IL2RB CD274 CP PNPO PDYN DAPK1 GORASP1 CDH5 FKBP1A
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 TRIM8 NPTXR HCAR1 SCN5A GATA4 VHL CSF2RB FGF23 PRSS1 PTGIS BCKDHB ERCC2
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