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A computationally inferred regulatory heart ageing model including post-transcriptional regulations

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Outline

- Goals & Motivations
- Methods
- Results and discussion
- Conclusions
- Future works

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Goals & Motivations

GOAL Computational modeling of heart senescence

- Semi-automated **workflow**
- Ability to identify a **regulatory model**
- **Data fusion** techniques coupled with **network analysis** theory
- Representation of the **relationship between key genes and miRNAs** involved in cardiac senescence processes

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Goals & Motivations

Why? Motivations

- **Cardiovascular diseases** are one of the leading causes of death
- **Ageing** is a dominant risk factor for their development
- **miRNAs** have been identified as relevant players in the development of cardiac pathologies
  - Inner ability to **influence gene networks**
  - Potential **therapeutic targets** or **diagnostic markers**

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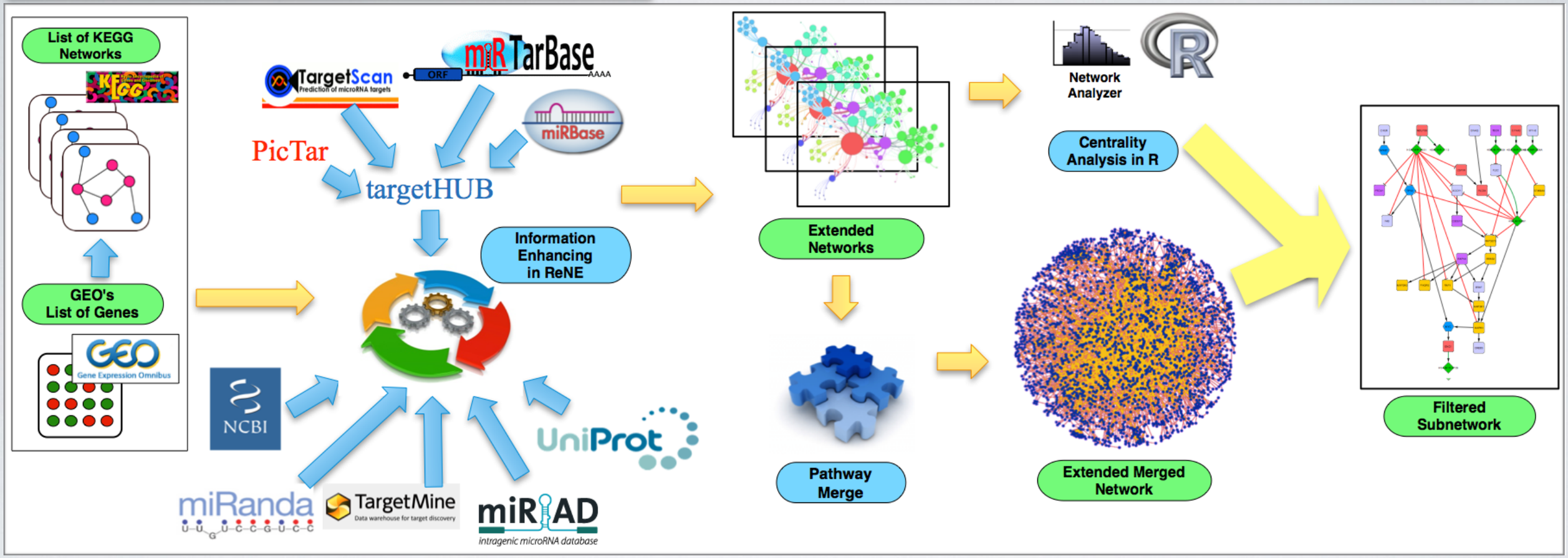
Methods

SEMI AUTOMATED PIPELINE



Data selection | **Data fusion** | **Data processing** | **Inference**

- Identifies key genes/miRNAs
- Creates a sub-network that dispatches key regulatory signals previously associated with senescence

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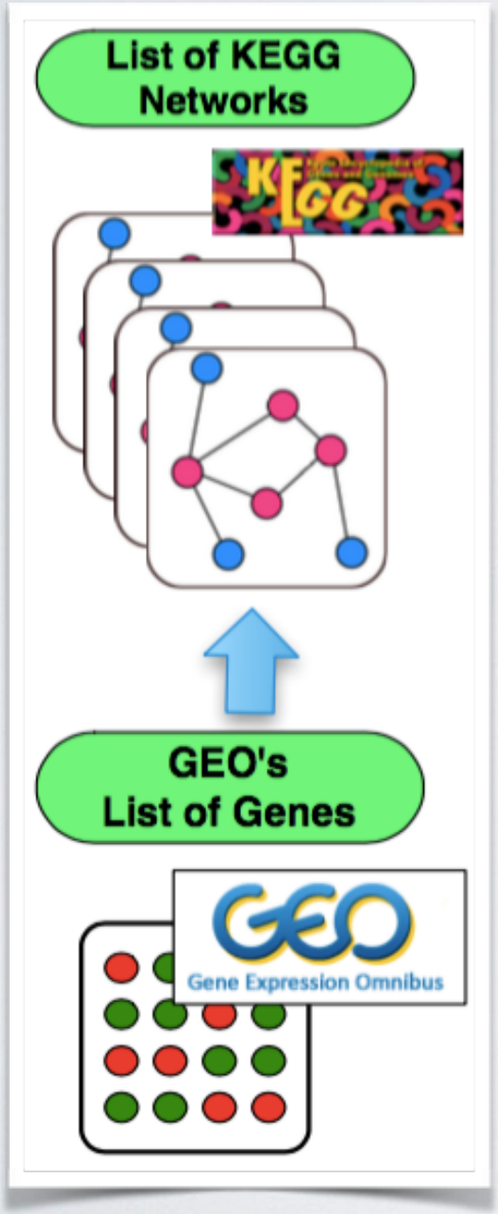
Methods

Data selection



- **A manually curated list of KEGG networks.**
  Networks ranked according to the presence of a given set of genes
  (top 15 pathways were analyzed)

- A **list of differentially expressed genes**, extracted from GEO and related to cardiac ageing.

  - Microarray data filtered using the GEO Differential Expression filter

  - Transcribed loci, pseudogenes, and expressed sequence tags removed from the obtained list

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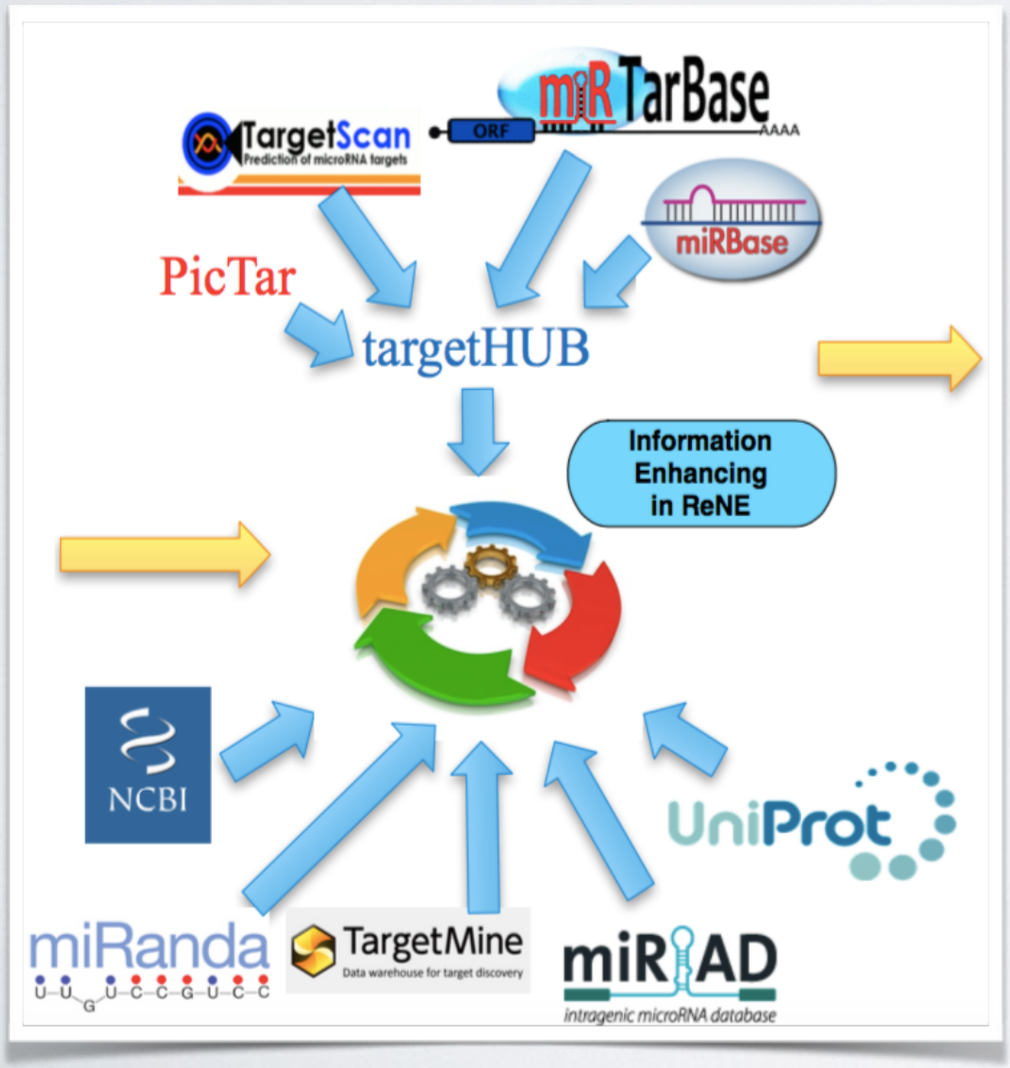
Methods

Data fusion

ReNE: A Cytoscape Plugin for Regulatory Network Enhancement
Politano G, et al. (2014), PLOS ONE



List of genes and selected pathways processed with **ReNE** Cytoscape plugin:

- **Naming normalization**: unique identifiers allow to navigate across public *-omic* repositories
- **Transcriptional enhancing**: interacting TFs retrieved from TargetMine and integrated in the network
- **Post-transcriptional enhancing**: intragenic miRNAs (hosted by the network genes) and intergenic miRNAs (co-expressed with their host genes) identified from the miRIAD database. The list of targets of each identified miRNA retrieved from TargetHUB

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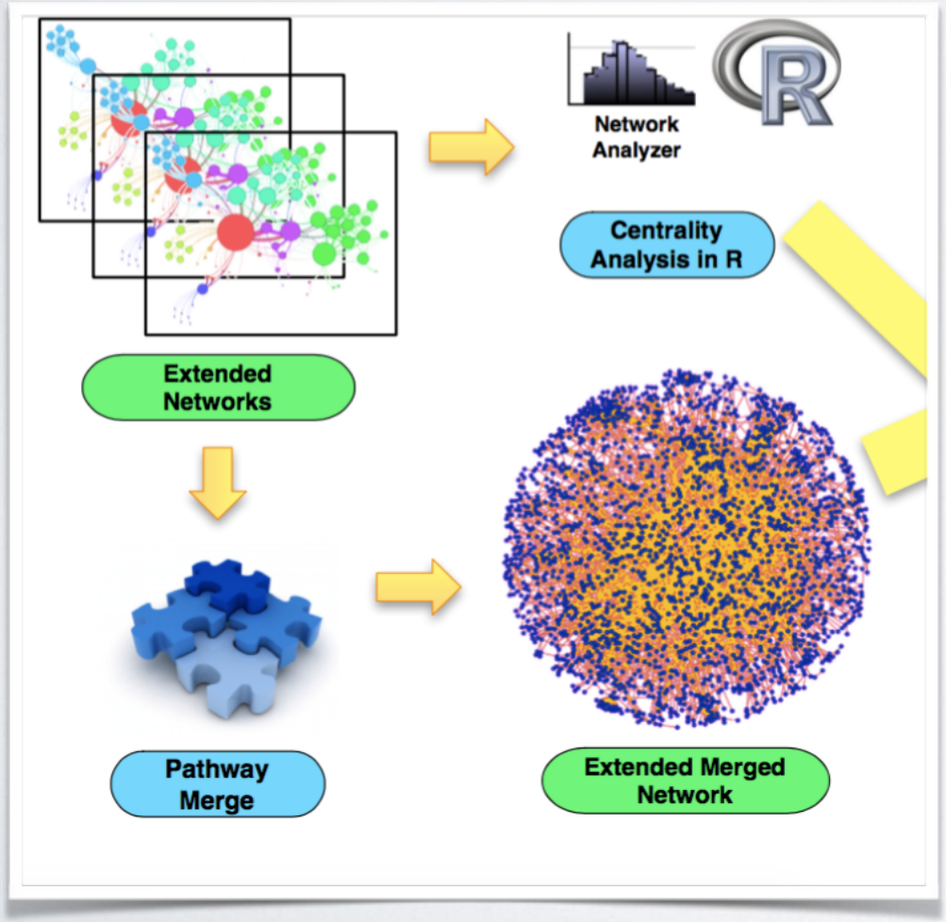
Methods

Data processing

Cytoscape **NetworkAnalyzer** used to compute **centrality measures** to identify key players in biological processes.
(**highly connected vertices** are often functionally important, their deletion lead to lethality)



- **Centrality measure**
  betweenness: quantifies the ability of a vertex to **monitor the communication** between other vertices

- **Post processing**
  A R script processes NetworkAnalizer results **in order to identify and to sort the nodes with the higher betweenness centrality**.
  (Produces a list of high centrality nodes that are promising regulators with a key role in signaling cascades related to aging)

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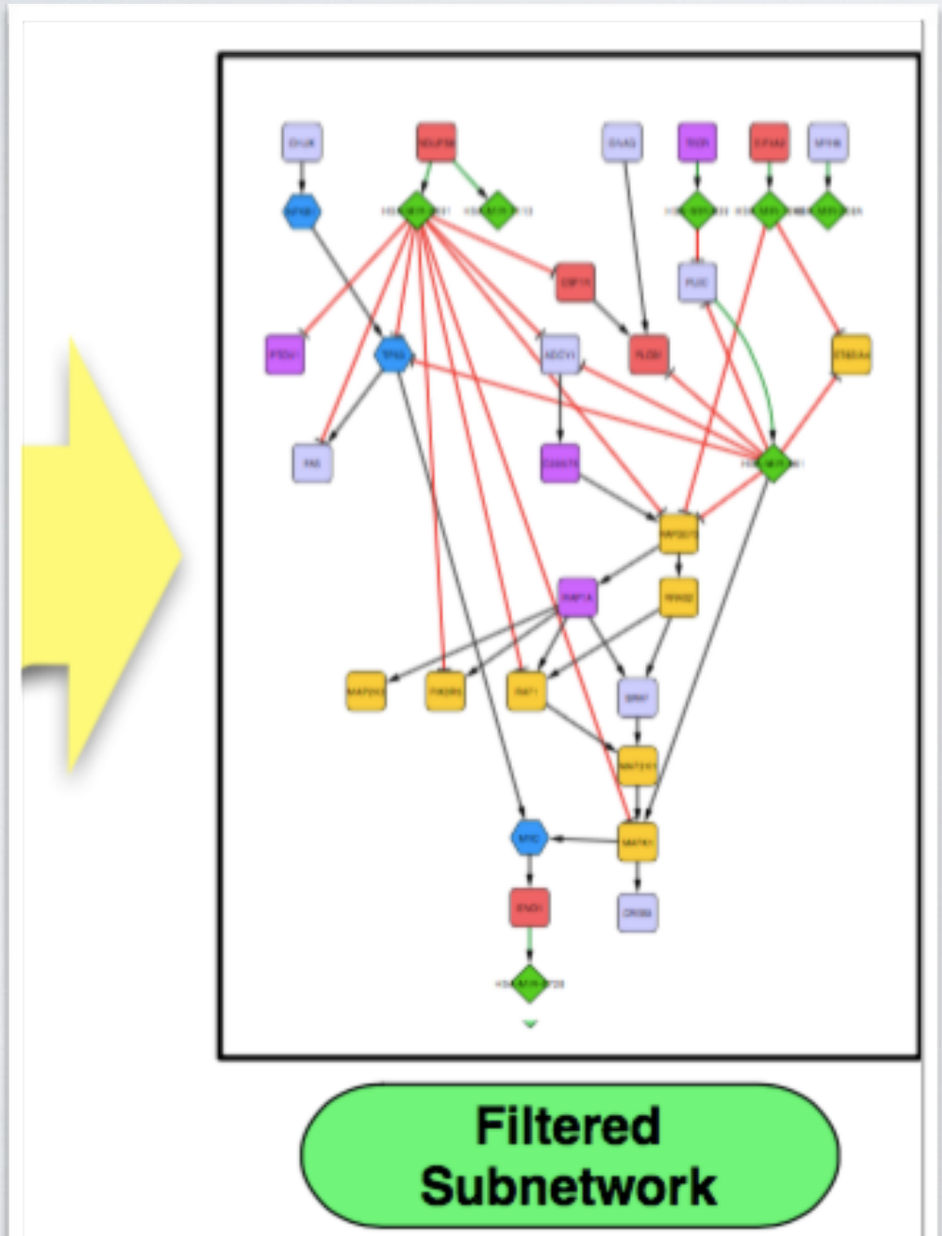
Methods

Inference



- The **ReNE Cytoscape** plugin used to merge all the pathways producing a **large network**.

- The network **filtered** in order to **retain only the high centrality nodes.**

- The filtered subnetwork is a good candidate to highlight the **most important inter-pathways regulatory entities**.

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Results

Experimental setup: Input I

DIFFERENTIALLY EXPRESSED GENES

- 8,799 microarray (*NCBI Geo: GSE421 Dataset*)
- 191 differentially expressed genes (GEO Differential Expression filter)
- 177 candidate genes after removing non-genes references
- 157 human homologs with a valid NCBI ID after naming normalization

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Results

Experimental setup: Input 2

KEGG PATHWAYS ranked according to these 157 genes.

The 15 top ranked pathways **manually analyzed,** selecting the following:

MAPK (*hsa04010*)

apoptosis, proliferation, survival, growth arrest, differentiation, motility, metabolism and senescence. **Alterations in ageing.**

RAP1 *(hsa04015)*

activated by shear stress, regulates NO production in endothelial cells. Deficiency leads to **endothelial dysfunction and hypertension**, common in the elderly

Neuroactive ligand-receptor interaction (*hsa04080*)

decrease in catecholamine-responsiveness in the elderly and its regulatory effect in **pathways disturbed in heart failure**

Results

Experimental setup: Input 2

KEGG PATHWAYS ranked according to these 157 genes.

The 15 top ranked pathways **manually analyzed,** selecting the following:

cAMP (*hsa04024*)

inhibition protects against heart failure and **attenuates heart ageing**

PI3K-AKT *(hsa04151)*

attenuation contributes to **age related changes in myocardium**
(cardiomyocyte hypertrophy, energy production, contractility, and stress response)

Alzheimer's disease and Huntington's disease (*hsa05010, hsa05016*)

protein misfolding in cardiomyopathies appear linked to neurodegenerative disorders

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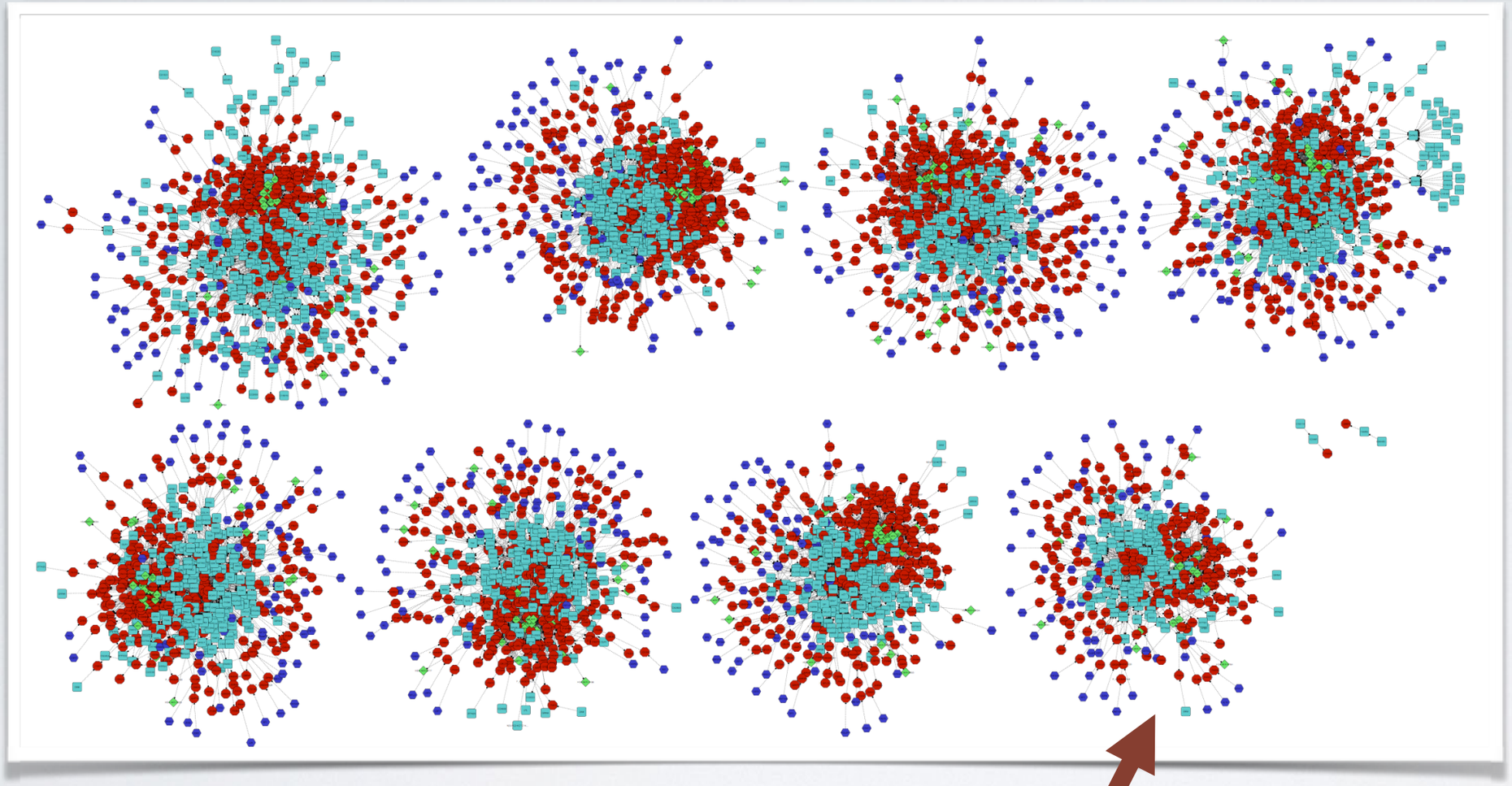
Results

Processing



- 7 pathways
- 157 genes

157 differentially expressed genes connected and transformed into a new pathway

ReNE: A Cytoscape Plugin for Regulatory Network Enhancement
Politano G, et al. (2014), PLOS ONE

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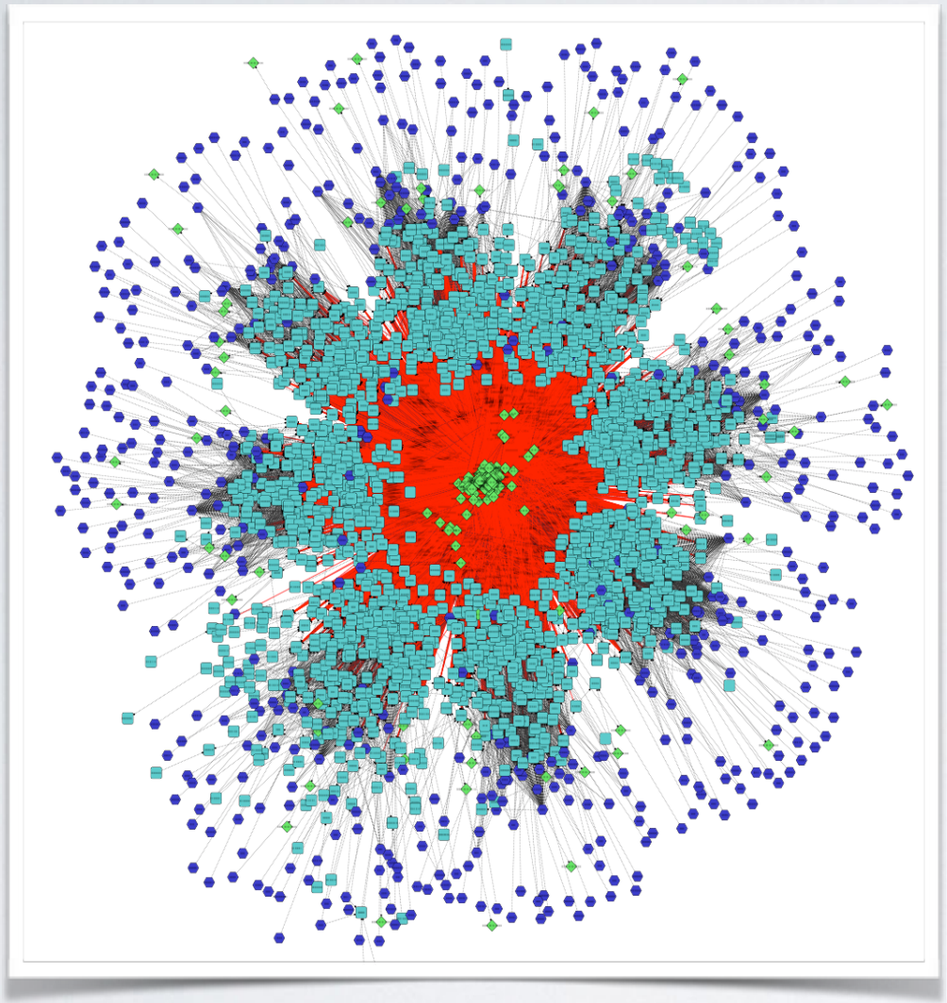
Results

Processing II

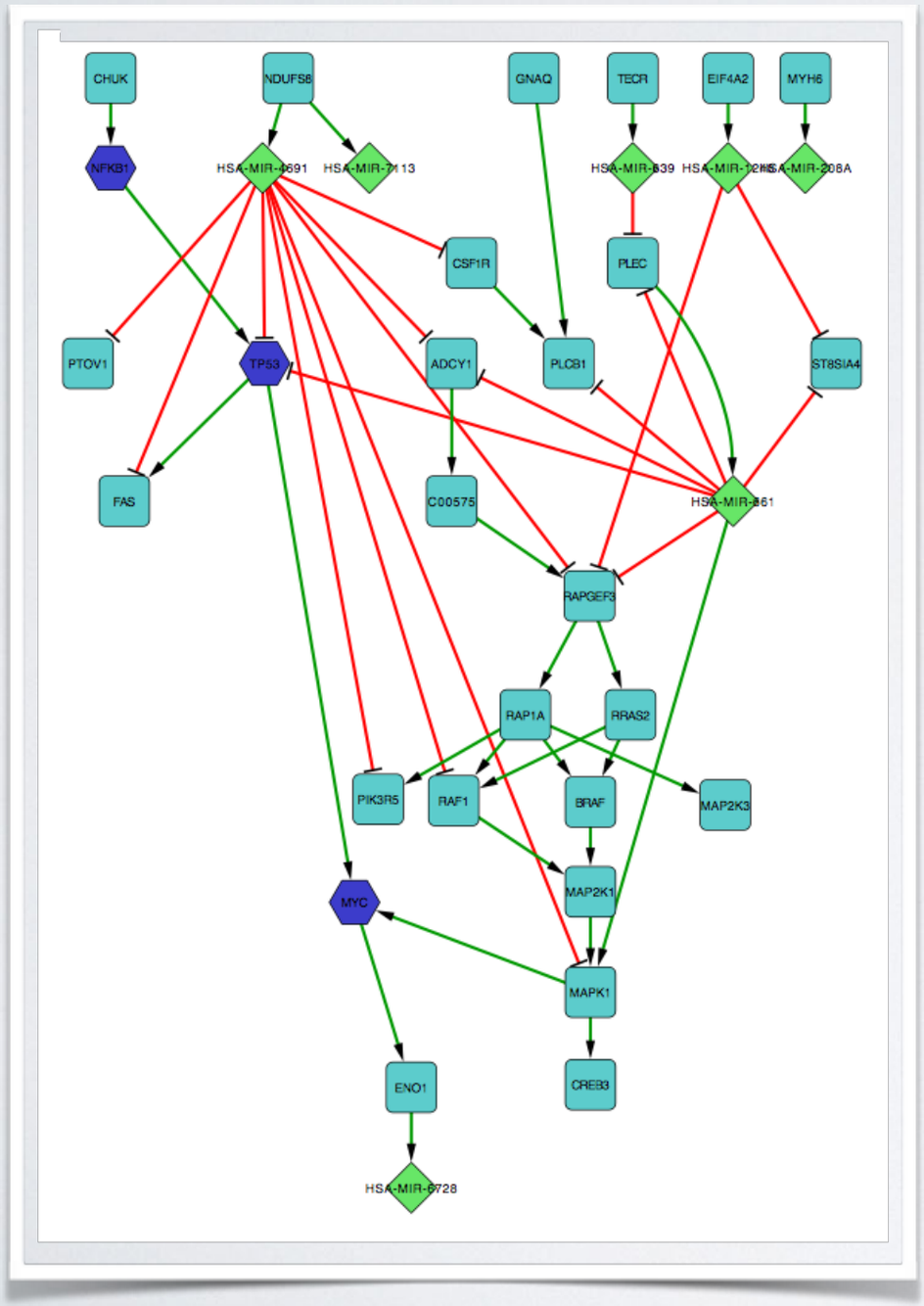


- **8 pathways merged**
  - 909 nodes (genes, TFs, and, miRNAs)
  - 6,475 edges
- **Pathways analyzed** with NetworkAnalyzer
  - Nodes Betweenness Centrality measured
  - Genes sorted accordingly
  - Top40 nodes selected (*high centrality nodes)*

<u>High Centrality Nodes</u>:
nodes with a probable **key role** in signalling cascades related to ageing. Their malfunctions can lead to widespread **functional misbehavior** of the entire regulatory network

Results

Processing III



- **Network filtered** according to the set of previously identified high centrality nodes
  - **35 nodes** sub-network
  - **New**: 3 TFs, 7 miRNAs
- Heart ageing signal cascades **regulatory model**

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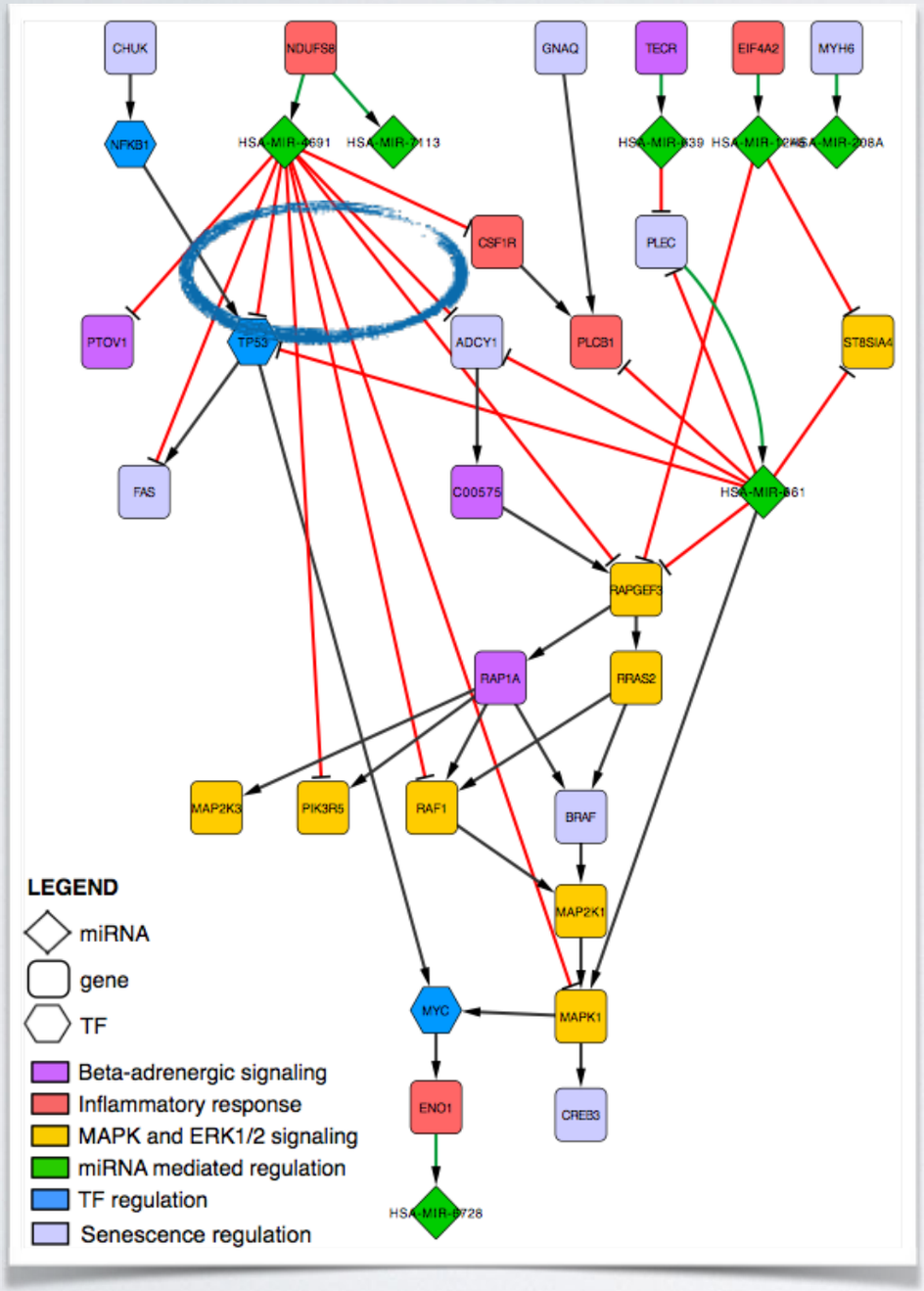
Discussion

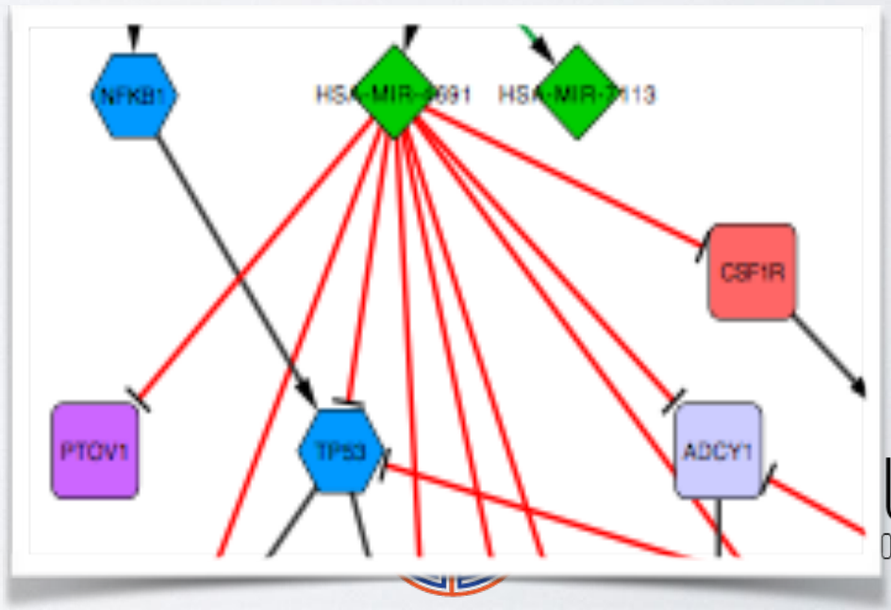


Literature validation

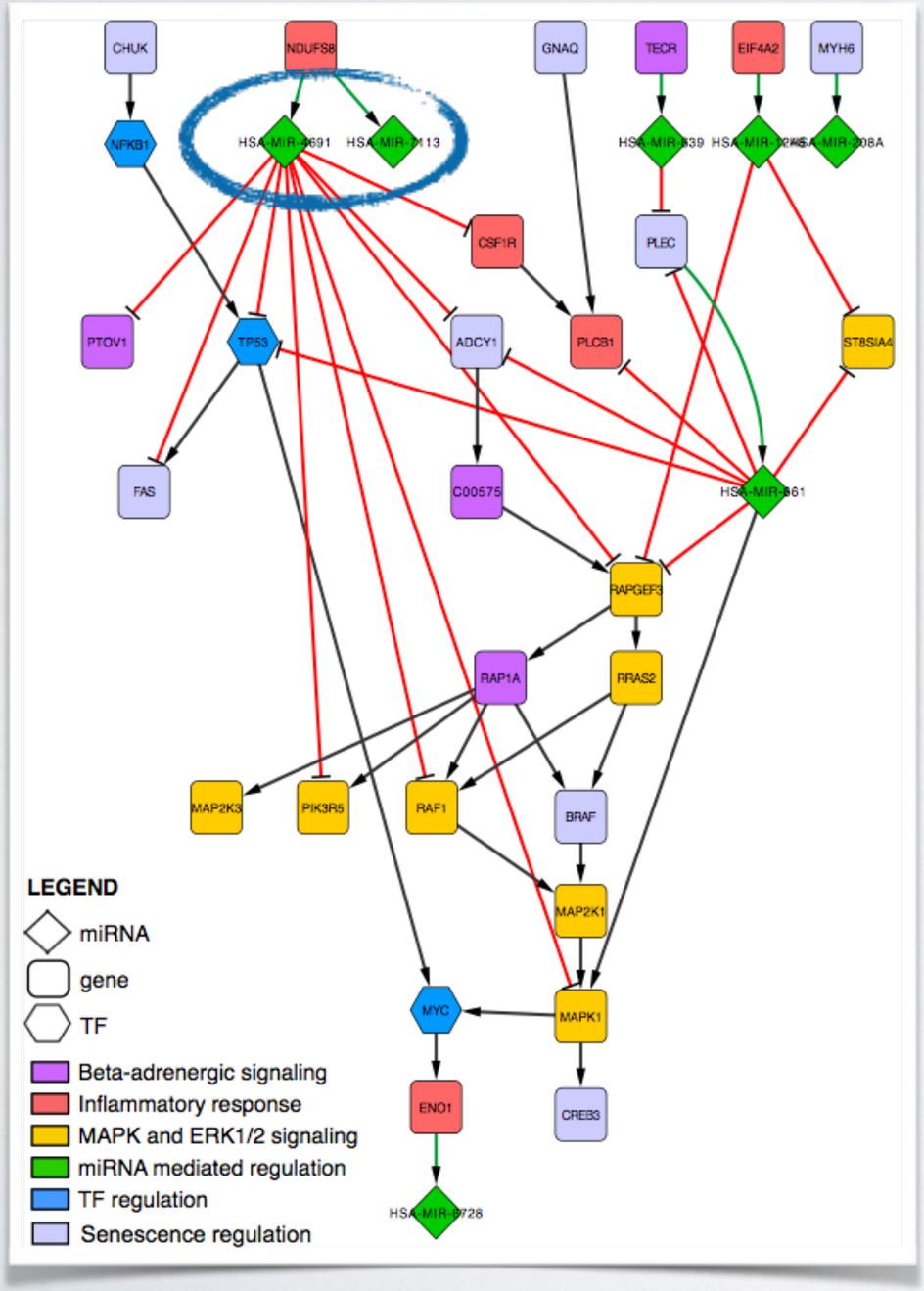
- nodes involved in ageing and senescence mechanisms

Non-validated Nodes

- miR-4691 (**regulatory hub, 9 regulated nodes**)
- miR-7113
- overall 33 out of 35 (94%)



Discussion



Classes:

- inflammatory response
- senescence regulation
- beta-adrenergic signaling
- MAP2K and ERK1/2
- miRNAs
- TFs

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Discussion

Beta-adrenerigc signaling

- **deleterious to heart:** increased heart rate, vasoconstriction and wall stress

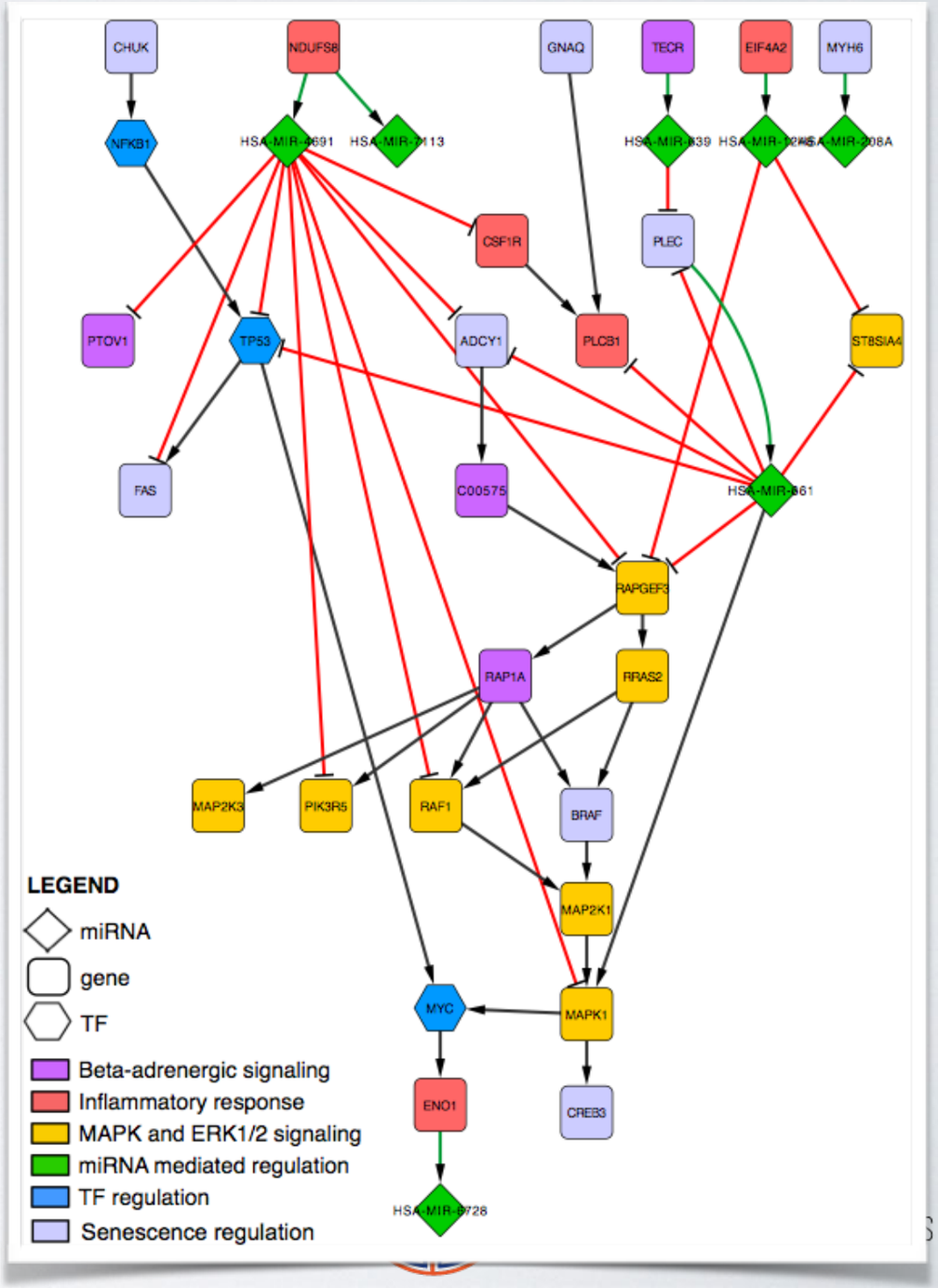
Androgens may drive myocardial diseases: atherogenic effects on the cardiovascular system, promote plaque formation and enhance monocyte adhesion to endothelial cells.

PTOV1: plays an important role in **androgen-related atherogenesis** in the male human aorta

TECR: candidate as processor for adrenergic and hormonal signals, capable to possibly lead to **vascular membranes impairment**

C00575 (cAMP): regulates the function of ion channels and a few other proteins such as RAPGEF3 (Exchange protein) and **RAP1**

RAP1: strengthens cell junction, reduces cell migration and protects telomeres. **RAP1 role in delaying senescence** and protecting DNA make it (and its regulators) good candidates for managing senescence



Discussion

Inflammatory response

- Senescence mechanisms driven by crosstalks with the ATM, Akt, and mTOR signaling pathways

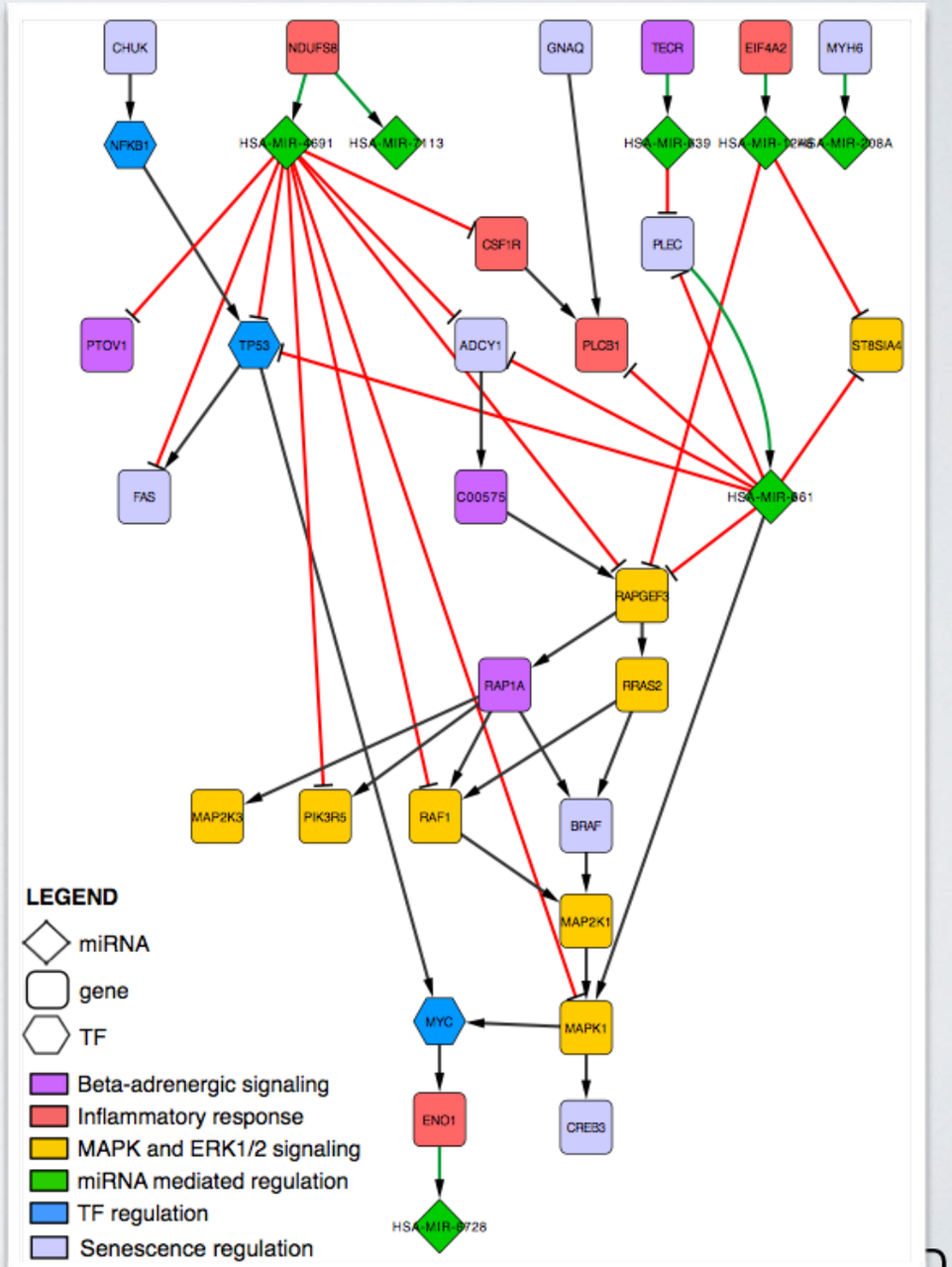
NDUFS8: mTORC1 downstream regulated gene and its presence may be an hallmark of mitochondrial regulation (**pro-inflammatory and pro-oxidant features of senescence**)

EIF4A2: influences protein translation, mitochondrial translocation efficiency, DNA recombination, replication, repair, transcription and **telomere maintenance**

ENO1: a common marker of systemic autoimmune diseases and inflammatory, degenerative and other pathological disorders. It is **induced by cellular stress and promote cell growth, glycolysis, migration, and invasion**

CSF1R: linked with AKT and physical crosstalk with PI3K, AKT, STAT3, and NFKB pathways. Proliferation, differentiation, recruitment, and survival of the monocyte–macrophage

PLCB1: pro-inflammatory regulator involved in several cardiovascular diseases (vasculitis, Kawasaki, endothelial cell inflammation, and coronary artery aneurysm)



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Discussion

Senescence regulation

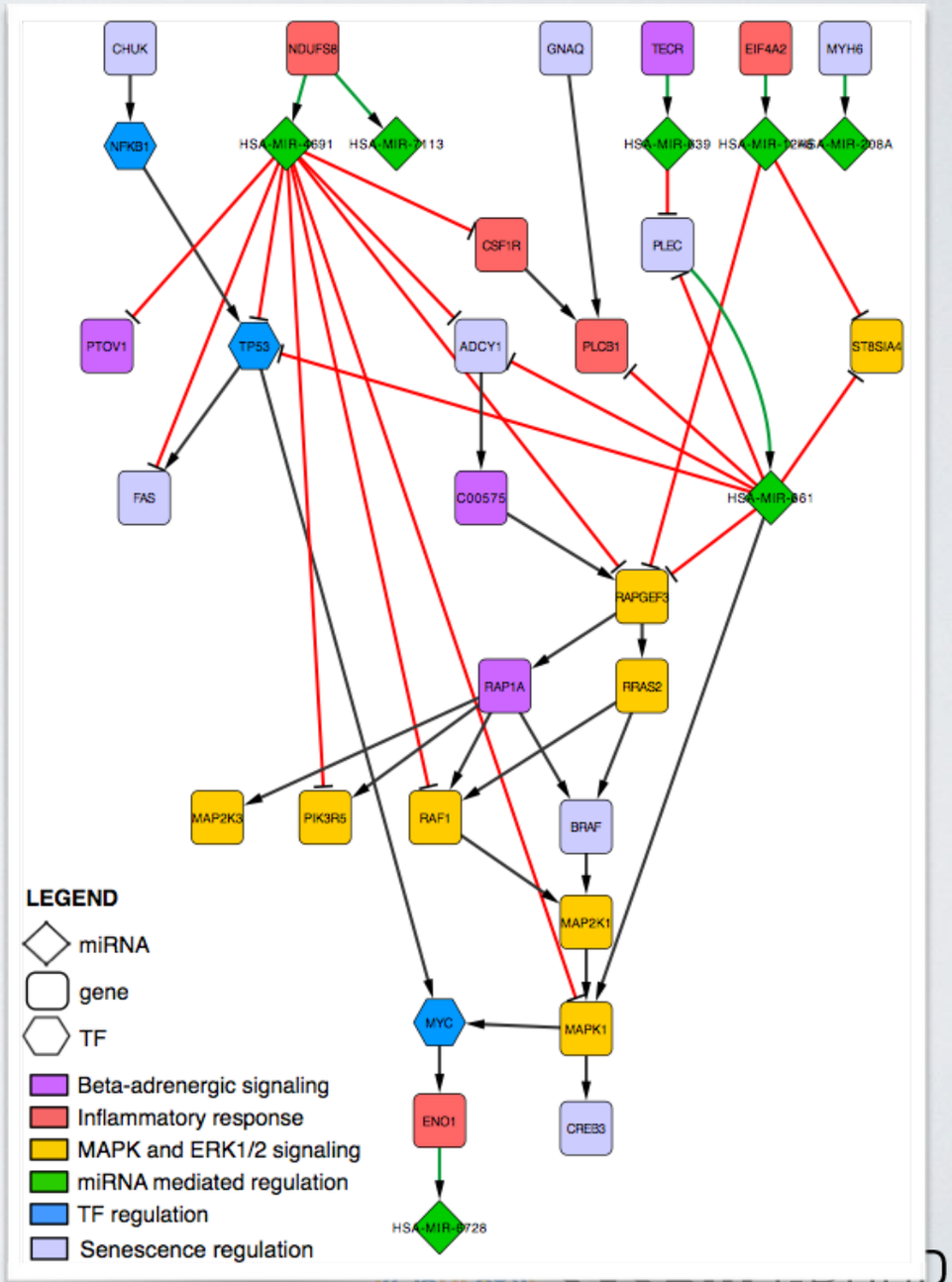
- Endothelial cell senescence seems to increase the incidence of cardio vascular disorders

PLEC: actin binding protein **induced in endothelial cells exposed to LSS** (laminar shear stress - regulate endothelial function and vascular health)

BRAF: its activation lead to the **proliferation and maturation/senescence of melanoblasts** (which eventually colonise the valves of the heart)

GNAQ: responsible (with NRAS/BRAF-mutations) for transcriptional modifications that lead to **melanoblast proliferation**

CREB3: a regulator, **ectopically expressed in abnormal lysosomes,** characteristic of cellular senescence, ageing, atherosclerosis, Alzheimer's



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Discussion

Senescence regulation

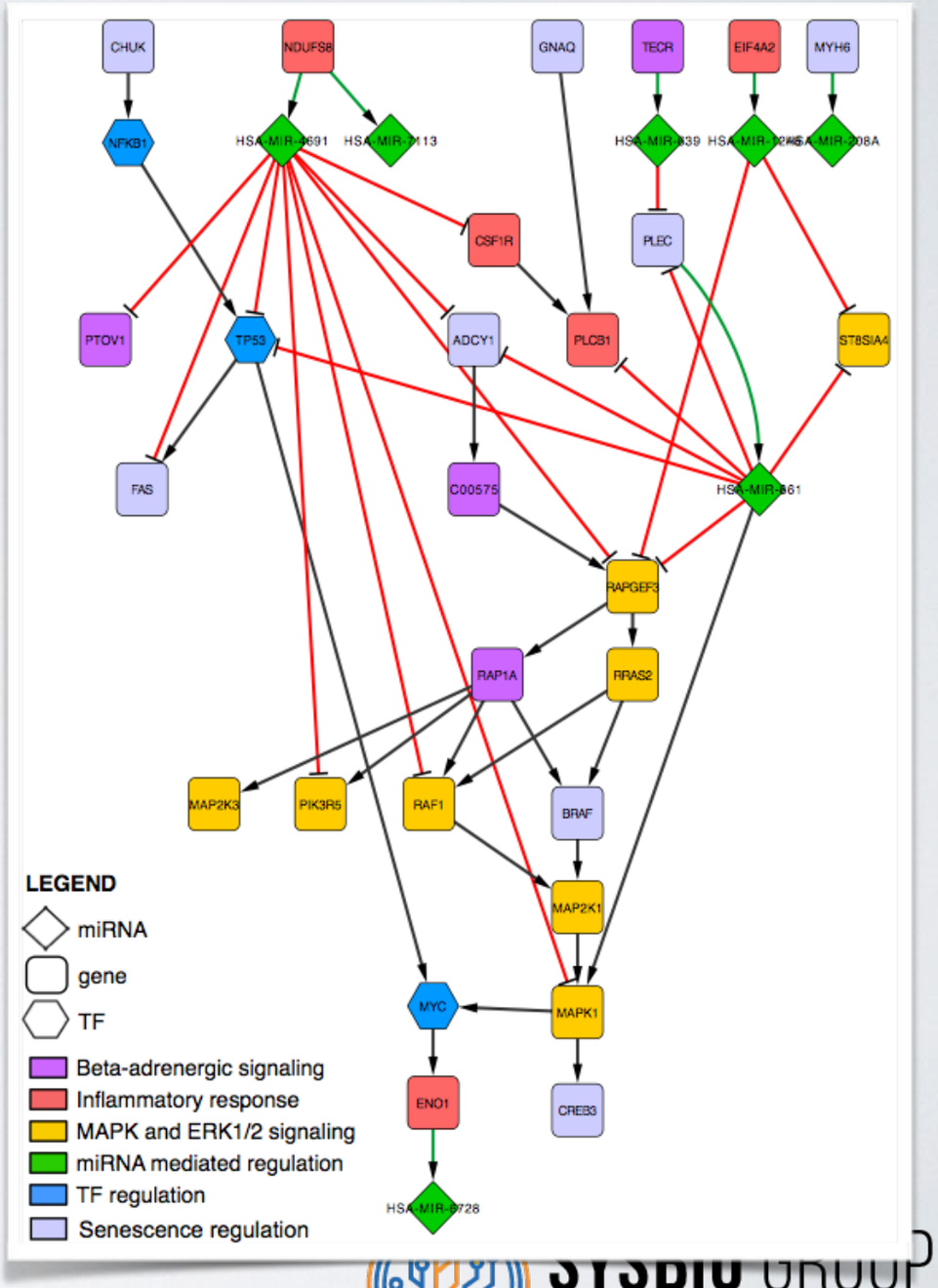
- Endothelial cell senescence seems to increase the incidence of cardio vascular disorders

MYH6: ectopically regulated in **senescence**

FAS: activates NFKB/MAPK/ERK/JNK pathways. It's impairing eads to **reduced immune function** and senescent cell accumulation in elders

ADCY1: associated with ERK may contribute to cAMP elevation in vascular smooth muscle cells. **cAMP over expression in the plasma membrane results in cardiac rejuvenation**

CHUK: **inhibitor of the transcription factor NFKB** complex. Reduced inhibition of NFKB is associated with the pro-inflammatory phenotype in vascular endothelium



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Discussion

MAP2K and ERK1/2

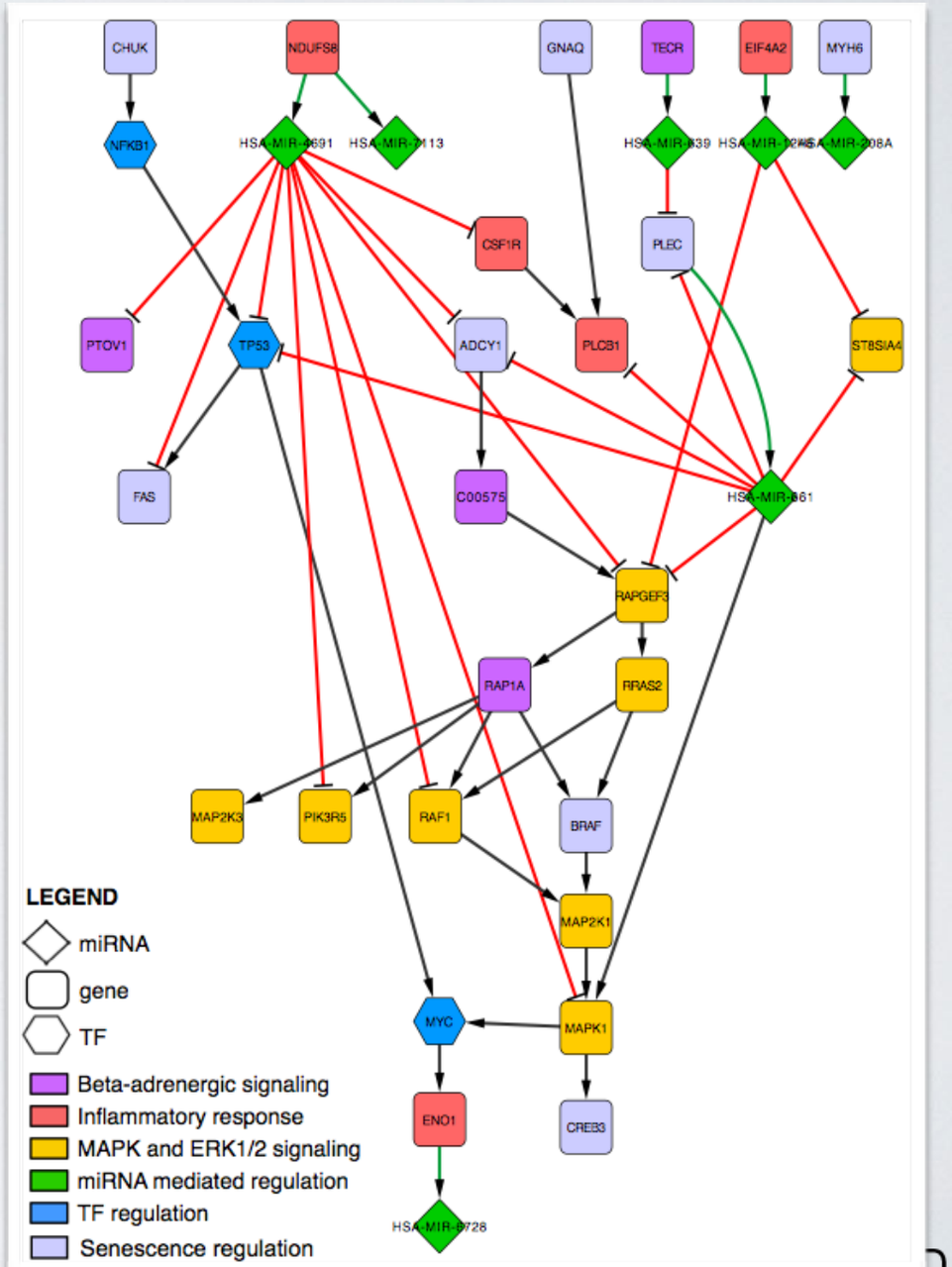
- β-adrenergic signals regulate cAMP, which in turn regulates crucial cell-survival pathways

Ras, Raf, MAP2K, and, ERK1/2 manage cell cycle regulation, **apoptosis, cell survival, senescence**, differentiation, and cell growth and migration.

RRAS2: involved in diverse processes including angiogenesis, vascular homeostasis and regeneration, and cell adhesion. **RAS proteins interact with RAF, PI3K and RAS/MAPK pathways** (cellular proliferation and differentiation)

ST8SIA4: substrate recognition that **modulates cell adhesion and signaling** (mediates activity of PI3K/Akt)

RAPGEF3: **protects cardiomyocytes** from oxidative stress and apoptosis, its ectopic expression may result in cell aging effects



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Discussion

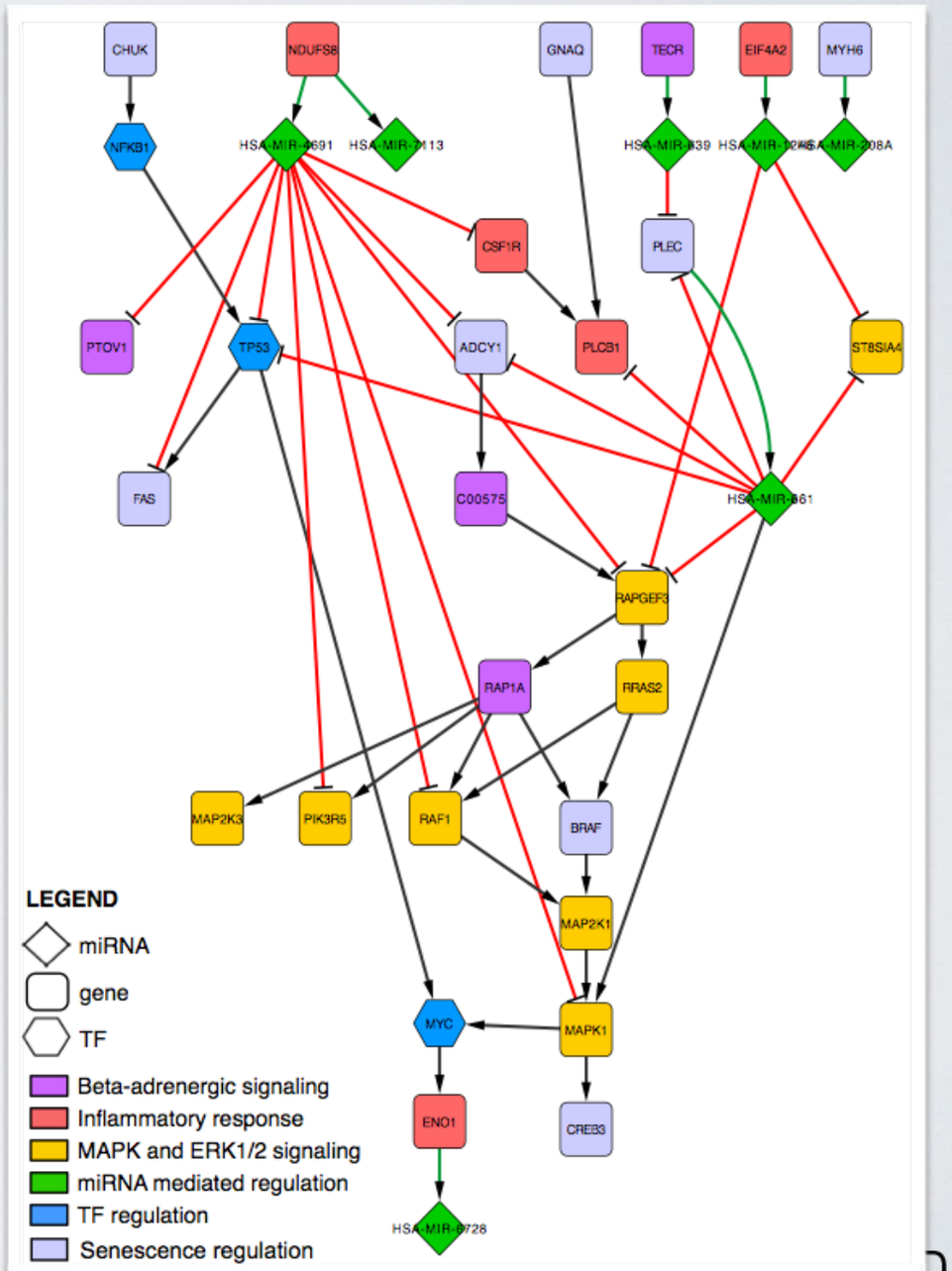
MAP2K and ERK1/2

- β-adrenergic signals regulate cAMP, which in turn regulates crucial cell-survival pathways

RAF1: its **deletion leads to heart failure,** increases apoptosis. Growth factors activate the signaling cascade (Ras-Raf-MAP2K-ERK) -> cytoplasm/nucleus protein phosphorylation

PIK3R5: activates the Ras-Raf-MAP2K-ERK signalling cascade. Its **inhibition prevents** the expression of **cellular senescence** markers and most of the **age-related changes** of gene expression

MAPK1/MAP2K3/MAP2K1: the MAPK cascade (Ras-Raf-MAP2K-ERK) regulates cellular kinases, and nuclear TFs. Mutations in HRAS, KRAS, BRAF, and MAP2K are involved in **genetic disorders with cardiac developmental defects**



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Discussion

miRNAs

- miRNAs are post-transcriptional co-regulators of gene expression

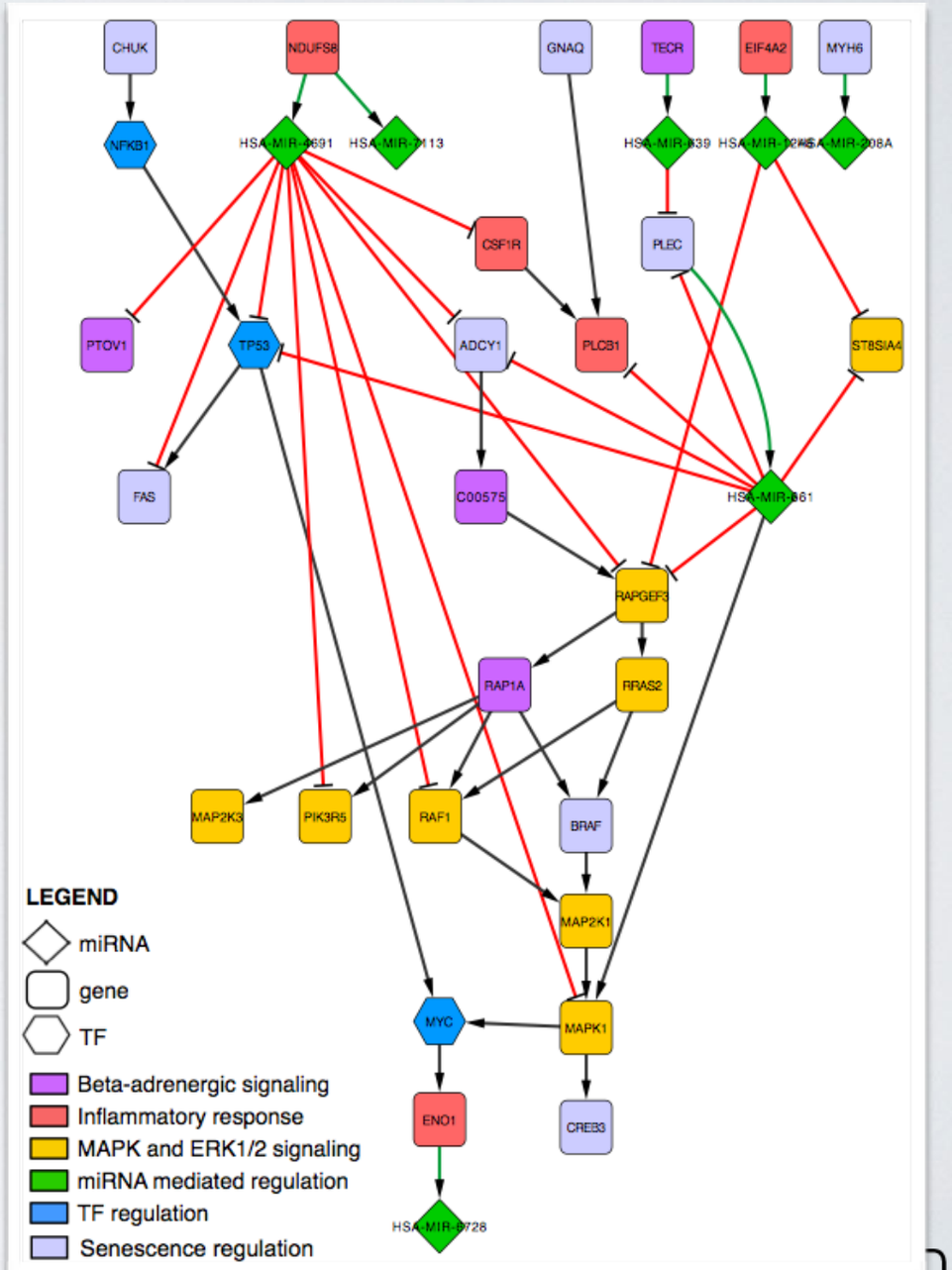
miR-6728: regulates several genes associated with **cardiometabolic** phenotypes

miR-639: novel biomarker of human bone marrow mesenchymal stem cells **aging**

miR-661: related to **inflammatory response**, associated with nano-sized vesicles released by activated T-lymphocytes

miR-1248: decreased in old participants (mean 64 yo), increased in young participants (mean 30 yo). Regulates several **age-associated cytokines** (IL6 and IL8) and **inflammatory-associated pathways** (including NF-kB)

miR-208a: its over expression induces **hypertrophic muscle growth and arrhythmias.** Given its high sensitivity, miR-208a seems a **reliable biomarker** for early acute myocardial infarction (AMI) diagnosis



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Discussion

Transcription factors

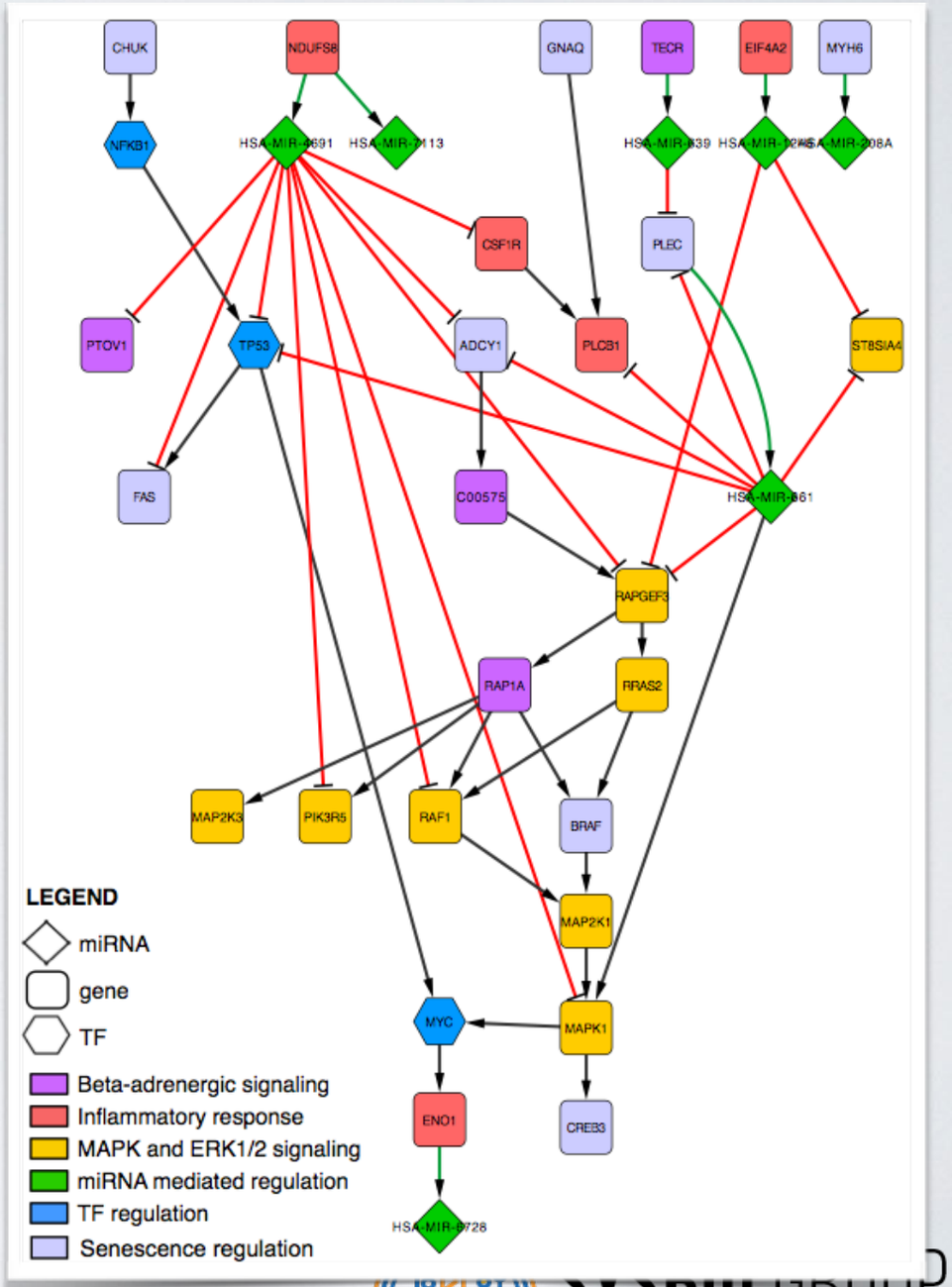
- TFs actively regulate the gene expression

MYC+TP53 lead to heart failure phenotype: enlarged myocytes, reduced number of cardiomyocytes, telomere attrition, up-regulation of molecular markers of senescence, and decline in cardiac function

TP53: encodes a tumor suppressor protein, that **promotes senescence as mechanism to prevent cancer**. Its over-expression induces cell cycle arrest, apoptosis, senescence, DNA repair, changes in metabolism

NFKB1: hub. It's associated with **inflammatory diseases** and inappropriate immune cell development. The **NFKB1** subunit, in particular, is responsible for **proinflammatory arterial phenotype developed with aging,** and is associated to vascular dysfunctions due to impaired nitric oxide processing

MYC: cell cycle progression, apoptosis and cellular transformation. MYC is the key regulator of **Nucleostemin** (NS), which is essential to preserve the regenerative potential of aging stem cells by **antagonizing senescence**. Levels of MYC decrese in myocardial senescence along with elevated levels of TP53.



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Conclusions

- Computationally inferred **Heart Aging Model**
- to represent the **relationship between key genes, TFs and miRNAs involved in cardiac senescence processes**
- 25/25 genes, 3/3 TFs and 5/7 miRNAs appear **strongly related to ageing** after literature validation
- Non-validated miRNAs (miR-4691 and miR-7133) represent **good candidates for further studies** to elucidate their involvement in cardiac ageing
- On-going work: **wet-lab experiments** to validate the most promising interactions identified in the model

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Questions?

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