



PLATFROM BASIC AND APPLIED OMICS TECHNOLOGIES

Research topics

Institute of Biology and Immunology of Reproduction

Bulgarian Academy of Sciences

- **Genomic and transcriptome analysis for functional assessment of global alterations in cell signaling**

One of the most advanced and emerging trends in the field of analysis of changes in cell signaling and the identification of changes in cell behavior is the study of their global transcriptome. The application of 3rd generation sequencing methods allows the evaluation of very long sequences by switching transcriptional isoforms. Another advantage of long sequencing is the ability to identify various de novo fusion phenomena associated with oncogenesis. The technology allows analysis of DNA modifications directly without preliminary bisulfide conversion.

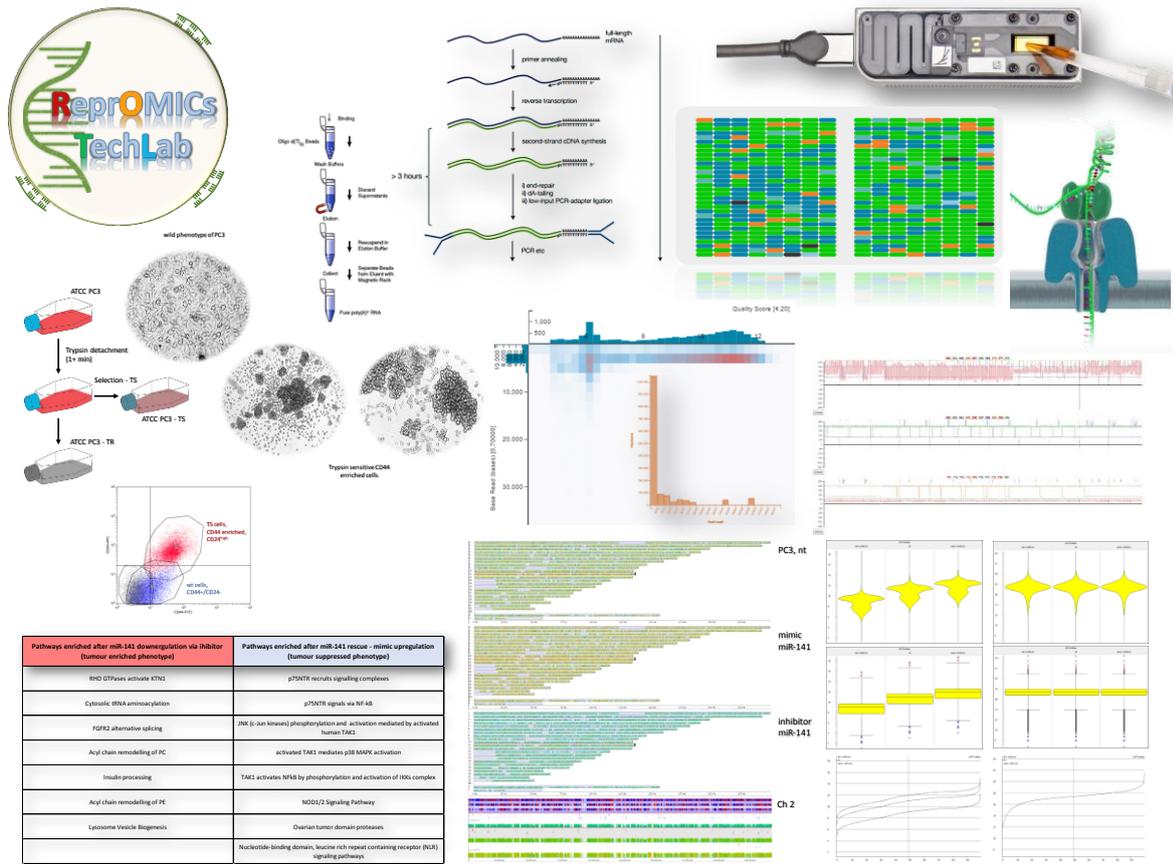


Figure 1. Diagram of selection of prostate cancer cells with enriched cantilever properties, mRNA isolation, library preparation, and sequencing using the MinION sequencer of Oxford Nanopore. Graphs show direct sequencing of long-length mRNA targets, normalization of data, mapping to the genome reference genes whose mRNAs are enriched, and results of analysis of changes in signaling pathways in cells that are only enriched in the stem, or are artificially inflated or with recovered micro-RNA-141. Logo of the Laboratory at IBIR (top left), photo of the sequencer and mechanism of action of the nanopore (top-right, ref. Nanoporetech).

The research team of the Laboratory of Reproductive OMICs Technologies of IBIR (Prof. S. Hajrabedian, DSc, Prof. K. Todorova, DSc) is a pioneer in the country in the implementation of a third-generation sequencing technology based on nanopores, successfully tested in 2016 by NASA at the International Space Station as the first space sequencing technology. The IBIR team implemented the technology for sequencing the complete transcriptome (all the information RNAs with which the cell encodes its proteins) of Sertoli cells - involved in the maturation and maturation of germ cells and in cells isolated from prostate carcinoma metastasis. In prostate cancer research, the researchers track those groups of cells that have the highest degree of loss of ability to participate in prostate tissue formation by acquiring properties of stem, undifferentiated cells. There is a disordered regulation of the non-coding micro-RNA-141, which is suppressed, as it also regulates cellular "self-sacrifice" (autophagy). Global changes in the expression of genes in cells in these more malignant versions of prostatic carcinoma cells and the role of this micro-RNA for their intracellular signals through nanoporous sequencing are tracked and the data show suppression of signals in the cell associated with congenital immune signaling (NOD family), transcriptional pro-inflammatory factors, neutroporin tumor suppressor, etc., and enhancement of signals associated with cell growth activation and multiplication by growth factors and hormones. The introduction of the nanoparticle sequencing is one of the reasons why Professor Hayrabedian was nominated in

2018 for the Pythagoras Prize for an established scientist in the Medicine field. The data are received on the basis of the financial support of the Bulgarian Academy of Sciences program for support of young scientists (projects DFNP 17-141, DFNP 17-142).

Activities and scientific expertise:

1. Isolation of mRNA and conversion to cDNA, sequence libraries, real-time sequencing of the transcript, basecalling, bioinformatic sequence processing - alignment and mapping, differential expression at the gene /transcript/ isoform level,
2. Optimization of methods for enrichment of cellular subpopulations with specific properties
3. DNA isolation, sequence libraries, real-time sequencing of the transcript, basecalling, bioinformatic sequence processing - alignment and mapping, analysis of changes in DNA methylation (global DNA methylation)
4. Bioinformatic analyzes to assess the correlation between gene expression, transcription isoforms, functional studies, and post-transcriptional modifications. Analysis of changes in signaling pathways.

Technical capacity:

- DNA/RNA Quality Assesment, quantitative Research, quality of sequencing libraries
- Real-time sequencing

Team:

Prof. Soren Hayrabetian; Prof. Krassimira Todorova; Prof. Ilka Tsvetkova; PhD student Albena Apostolova; PhD student Leila Askova; PhD student Radostina Tsvetankova;

Relevant publications:

1. **Krassimira Todorova, Metodi V Metodiev, Gergana Metodieva, Milcho Mincheff, Nelson Fernandez, Soren Hayrabetian.** *Micro-RNA-204 participates in TMPRSS2:ERG regulation and androgen receptor reprogramming in prostate cancer. Hormones and Cancer, 8(1):28-48, 2017 Jan 3. doi: 10.1007/s12672016-0279-9, IF=3.167*
2. **Soren Hayrabetian, Krassimira Todorova.** *Recent Trends in Cancer Biology: Spotlight on Signaling Cascades and microRNAs. Cell Signaling Pathways and microRNAs in Cancer Biology. Chapter 14. "When the Molecules Start Playing Chess, or How MicroRNAs Acquire Dualistic Activity During Cancer Progression."* 1, Springer-Nature International Publishing AG, 2018, ISBN:9783-319-71552-0, DOI:10.1007/978-3-319-71553-7
3. **K. Todorova, E. Avramaska, L. Sezer, A. Apostolova, S. Hayrabetian.** *P.C1.08.08. Sertoli cells have non-canonical functional inflammasome network able to perturb testis niche immune tolerance and to inflict cell death. 5th European Congress of Immunology - ECI 2018 – 2-5 September, Amsterdam, The Netherlands*

4. **Hayrabyan S., Todorova K.** Advantages of Oxford Nanopore native molecule sequencing for personalized diagnostics and its application in Sertoli cell transcriptomics for innate immunity and inflammasome signaling research. 15th International Symposium for Immunology of Reproduction. "50 Years of International Coordination Committee for Immunology of Reproduction", 15–17 June, 2018, Varna, Bulgaria
5. **Hayrabyan S.** Advantages of native nanopore sequencing (Oxford Nanopore) for personalized diagnostics. 40 years sequencing - a new era in contemporary diagnostics and personalised medicine. Personalized Medicine School organized by the Bulgarian Association for Personalized Medicine (BAPEMED) and Medical University of Plovdiv, Plovdiv, Bulgaria, 3-7 Nov, 2017
6. **Apostolova A., Hayrabyan S., Todorova K.** Hsa-miR-141 whole transcriptome changes in prostate cancer metastasis with stem cell enriched phenotype – seeking for new markers for personalized medicine. 15th International Symposium for Immunology of Reproduction, 15–17 June, 2018, Varna, Bulgaria
7. **Soren Hayrabyan, Krassimira Todorova,** Complex inflammasome signalling in Sertoli cells revealed, following innate immunity challenge in DAMP associated context. Implications for male infertility, Fifth National Congress of Immunology „Immunology for All”, 25 – 28 October 2018, Imperial Hotel & SPA, Plovdiv
8. **Leyla Sezer, Elina Avramaska, Krassimira Todorova, Soren Hayrabyan,** Innate immunity Tlr4 and Nod1 receptor signaling modulates Sertoli cells inflammasome and metabolic profile. Fifth National Congress of Immunology „Immunology for All”, 25 – 28 October 2018, Imperial Hotel & SPA, Plovdiv
9. **Sezer L., Avramaska E., Todorova K., Hayrabyan S.** Innate immunity TLR4 receptor signaling modulates Sertoli cells metabolic profile and induced NF-κB in MAPK1-dependent manner. 15th International Symposium for Immunology of Reproduction, 15–17 June, 2018, Varna, Bulgaria
10. **Apostolova A., Sezer L., Hayrabyan S., Todorova K.** MicroRNA15a could participate in prostate cancer progression by modulating cell proliferation and proinflammatory signaling. 15th International Symposium for Immunology of Reproduction, 15–17 June, 2018, Varna, Bulgaria
11. **Albena Apostolova, Leyla Sezer, Soren Hayrabyan, Krassimira Todorova.** The role of microRNA-15a in development of prostate cancer by modulating cell proliferation and pro-inflammatory signalling. *Acta Medica Bulgarica*. Vol. XLV, 2018, № 2, 20-24
12. **Canh P. Voong, Patrick S. Spencer, Cristina V. Navarrete, David Turner, Soren B. Hayrabyan, Philip Crummy, Emma Holloway, Mike T. Wilson, Patricia R. Smith, Nelson Fernández.** HLA-DR Genotyping and Mitochondrial DNA Analysis Reveal the Presence of Family Burials in a Fourth Century Romano-British Christian Cemetery. *Frontiers in Genetics*, 8, 182, Frontiers Media SA, 2017, DOI:10.3389/fgene.2017.00182, 1-10. ISI IF:3.789 C ISI IF - Q2

- **Bioinformatic analyzes of proteomics, transcriptomics, and immunological epitopes predictions for oncology and reproductive medicine. Generation of detection systems for fusion and other non-natural molecular configurations. Application of imaging methods and data mining to retrieve new biological phenomena of interaction**

A rapidly developing area is the analysis of proteomic and transcriptional data and the extraction of data for activation of cell signaling pathways. These analyzes lead to the

identification of target molecules or whole signaling pathways, and require subsequent validation of the mechanisms leading to these changes.

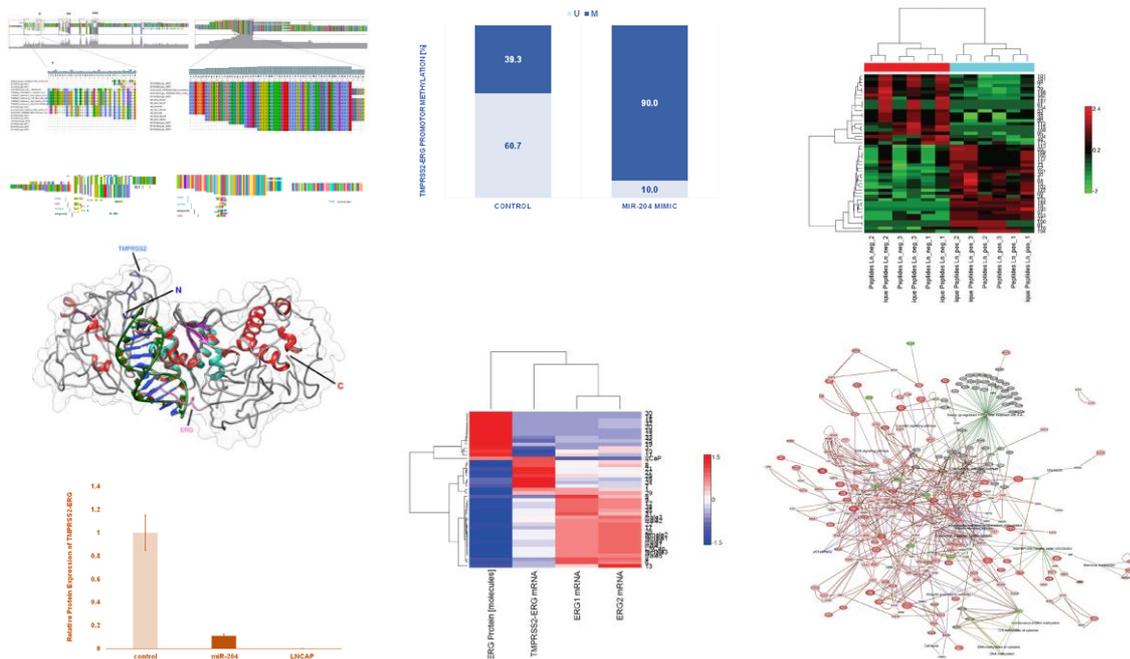


Figure 2. Sequences of the genes encoding *TMPRSS2* and *EGR* show specific mutant and normal-type sites, with locations of increased mutagenicity for recognition with semi-clonal antibodies. An *in-silico* model of various mutant variants was developed to determine the spatial location of the immunogenic sites. The change in the amount of protein encoded by the mutant gene, a *TMPRSS2* and *EGR* gene fusion product, was measured. *MiR-204* significantly reduces its levels by hypermethylation of its promoter. Mutant protein levels correlate with the levels of the mRNA encoding it, it is compensatory in larger amounts in patients without correctly folded protein. The analysis of proteomic alterations shows that *miR-204* replicates a number of target androgen receptor genes (Summary Collage of Figures from *HORM CANC* (2017) 8: 28-48 DOI 10.1007 / s12672-016-0279-9).

Activities and scientific expertise:

1. Analysis of gene-regulatory networks from experimental data from proteomics or transcriptomics and their relationship with reference annotations to other databases of co-expressed genes, mutations leading to diseases,
2. Analysis of the structure of fusion proteins *in silico* to evaluate target sites for detection using antibodies
3. Analysis of primary and secondary structure of polypeptide chains to identify potential epitopes for antibody detection in fusion gene products
4. Development of methods for the detection of fusion genes by means of a combination of antibodies and amplification of the signal by amplification of conjugated oligonucleotides (*TMPRSS2:ERG* patent application filed)
5. Metabolic Analysis of gene promoter methylation status
6. Functional analysis of innate immune response receptors
7. Analysis of functional status of inflammasomes in cells related to the reproductive system

Technical capacity:

- Analysis of gene-regulatory networks
- Analysis of the structure of fusion proteins in silico
- Development of methods for detecting fusion genes
- Protein study of fusion gene products (TMPRSS2:ERG patent application is currently being filed)
- Analysis of the methylation status of promoters

Team:

Prof. Soren Hayrabedian; Prof. Krassimira Todorova;

Relevant publications:

Hayrabedian, S, Todorova, K, Jabeen, A, Metodieva, G, Toshkov, S, Metodiev, M, Mincheff, M, Fernández, N. Sertoli cells have a functional NALP3 inflammasome that can modulate autophagy and cytokine production. *Scientific Reports*, 6, 18896, Nature Publishing Group, 2016, DOI:10.1038/srep18896, 1-17. ISI IF:5.578 C ISI IF – Q1

Todorova, K, Metodiev, M, Metodieva, G, Zasheva, D, Mincheff, M, Hayrabedian, S. miR-204 is Dysregulated in Metastatic Prostate Cancer In Vitro. *Molecular Carcinogenesis*, 55, 2, Wiley Periodicals, Inc., 2016, ISSN:1098-2744, DOI:10.1002/mc.22263, 131-147. ISI IF:4.808 C ISI IF – Q1

Todorova, K, Metodiev, M, Metodieva, M, Mincheff, M, Fernandez, N, Hayrabedian, S. Micro-RNA-204 participates in TMPRSS2:ERG regulation and androgen receptor reprogramming in prostate cancer.. *Hormones and Cancer*, 8, 1, Springer US, 2017, ISSN:1868-8497, DOI:10.1007/s12672-016-0279-9, 28-48. ISI IF:3.709 C ISI IF - Q2

- **In silico analysis of molecular interactions – peptide ligand – receptor protein**

Another very advanced and rapidly developing field is the in silico analysis of interactions between different peptide ligands and their receptors, the determination of functional mutants and the application of biophysical methods for validation of models.

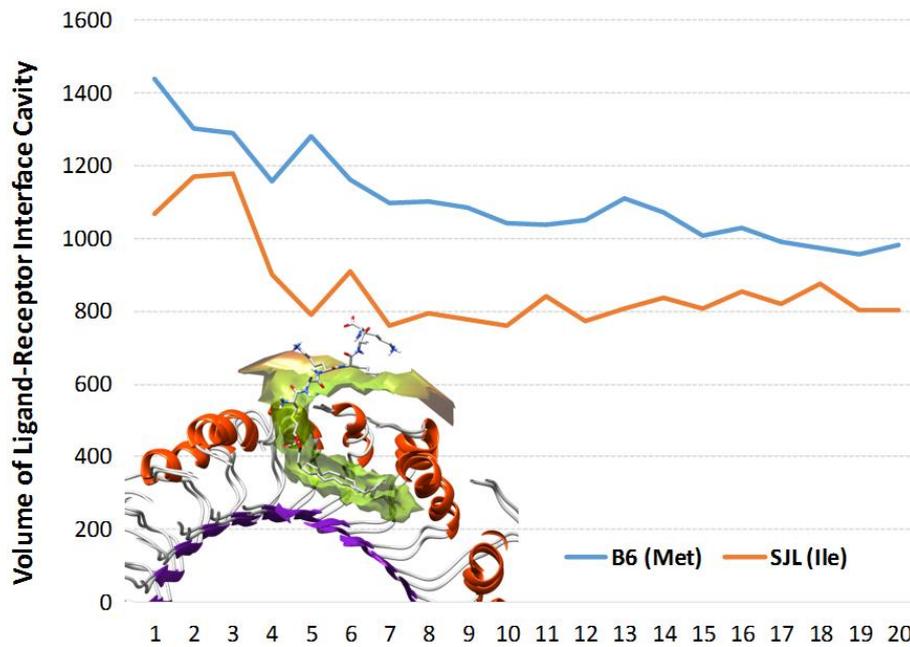


Figure 3. Molecular dynamics and ligand - receptor binding assays show that replacing Isoleucine with Methionine at the 82 position in the receptor molecule alters its tertiary structure, the volume of the "cavity" to which the peptidoglycan ligand PAM2CSK and the energy of connection, reducing the probability of connecting and conducting a signal. The "cutoff" of the binding is represented as a green interface, and the volumes of both the natural and the mutant receptor are represented in blue and red in the figure with a molecular pathway to minimize receptor energy. *Frontiers in Immunology* (IF 5.695): <http://journal.frontiersin.org/article/10.3389/fimmu.2016.00191/>

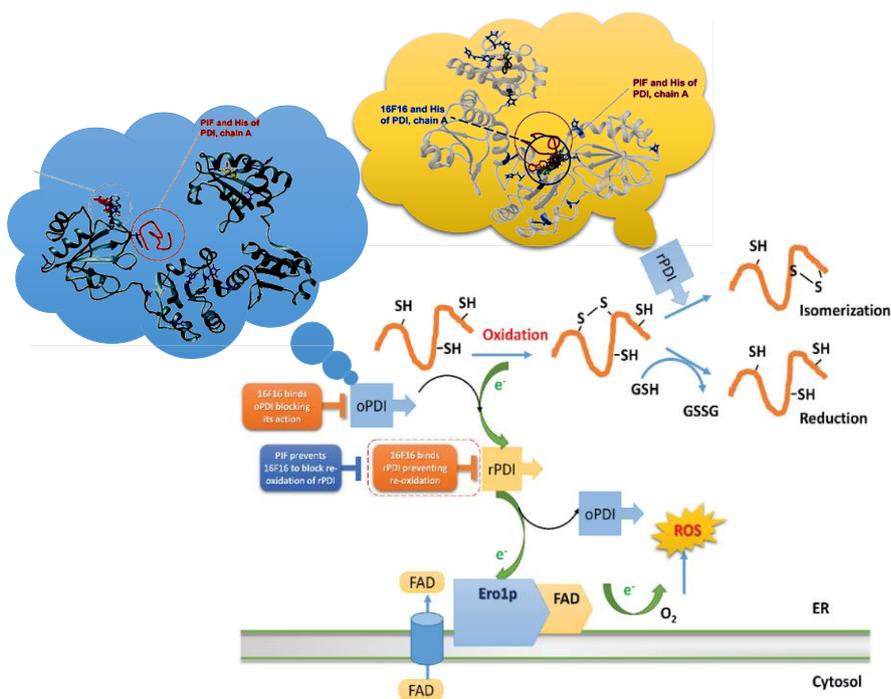


Figure 4. Mechanism of action of PIFtm showing blocking the possibility of production of reactive oxygen species and a structural model showing the spatial blocking of the enzyme inhibitor protein disulfide isomerase responsible for protection from oxidative stress and for proper coiling of the newly synthesizing proteins (*Oncotarget*, 2017, Vol. 8, (No. 20), pp: 32419-32432)a

Activities and scientific expertise:

1. In silico models of ligands, receptors and their interactions.
2. Analysis of interaction between ligands and receptors through different docking techniques
3. In silico mutagenesis to determine significant amino acid residues in peptide ligands
4. Molecular dynamic models for determining changes in receptors resulting from mutations and / or interactions with different ligands
5. Performing differential scanning fluorimetry to evaluate the degree of interaction at various ligand-receptor complexes

Technical capacity:

- Analysis of peptide ligand characteristics and mechanism of action
- Screening libraries from ligands to different target receptors
- Functional ion channel analyzes

Team:

Prof. Soren Hayrabedian; Prof. Krassimira Todorova;

Relevant publications:

Piermattei, A, Migliara, G, Di Sante, G, Foti, M, **Hayrabedian, S**, Papagna, A, Geloso, M, Corbi, M, Valentini, M, Sgambato, A, Delogu, G, Constantin, G, Ria, F. Toll-Like Receptor 2 Mediates In Vivo Pro- and Anti-inflammatory Effects of Mycobacterium Tuberculosis and Modulates Autoimmune Encephalomyelitis. *Frontiers in Immunology*, 7, 191, Frontiers, 2016, DOI:10.3389/fimmu.2016.00191, ISI IF:5.695 **C ISI IF – Q1**

Soren Hayrabedian, Krassimira Todorova, Marialuigia Spinelli, Eytan R. Barnea, Martin Mueller. The core sequence of PIF competes for insulin/amyloid β in insulin degrading enzyme: potential treatment for Alzheimer's disease. *Oncotarget*, 9, Impact Journals, LLC, 2018, DOI:https://doi.org/10.18632/oncotarget.26057, 33884-33895. SJR:1.942, ISI IF:4.67 **C ISI IF - Q1**

Goodale LF, **Hayrabedian S, Todorova K.**, Roussev R, Ramu S, Stamatkin C, Coulam CB, Barnea ER, Gilbert RO. Preimplantation factor (PIF) protects cultured embryos against oxidative stress: relevance for recurrent pregnancy loss (RPL) therapy. *Oncotarget*, 8, 20, Impact Journals, LLC, 2017, DOI:10.18632/oncotarget.16028, 32419-32432. ISI IF:5.168 **C ISI IF - Q1**

Chen, Y, Rivera, J, Fitzgerald, M, Hausding, C, Ying, Y, Wang, X, **Todorova, K, Hayrabedian, S**, Barnea, E, Karlheinz, P. Preimplantation factor prevents atherosclerosis via its immunomodulatory effects without affecting serum lipids. *Thrombosis and Haemostasis*, 111, 5, Schattauer Publishers, Stuttgart, 2016, ISSN:0340-6245, DOI:10.1160/TH15-08-0640, 871-1079. ISI IF:5.255 **C ISI IF – Q1**

Barnea, ER, Lubman, DM, Liu, YH, Absalon-Medina, V, **Hayrabyan, S, Todorova, K**, Gilbert, RO, Guingab, J, Barder, TJ. Insight into Preimplantation factor (PIF*) mechanism for embryo protection and development: target oxidative stress and protein misfolding (PDI and HSP) through essential RIPK binding site.. *PLOS One*, 9, 10, PLOS, 2014, DOI:10.1371/journal.pone.0100263, **ISI IF:3.534 C ISI IF – Q1**

Barnea, E, **Hayrabyan, S, Todorova, K**, Almogi-Hazan, O, Or, R, Guingab, J, McElhinney, J, Fernandez, N, Barder, T. Preimplantation factor (PIF) regulates systemic immunity and targets protective regulatory and cytoskeleton proteins. *Immunobiology*, 221, 7, Elsevier, 2016, ISSN:0171-2985, DOI:10.1016/j.imbio.2016.02.004, 778-793. **ISI IF:3 C ISI IF – Q2**