Omic evaluation of resilience and adaptation to the combination of heat and exercise stressors in horse athletes.

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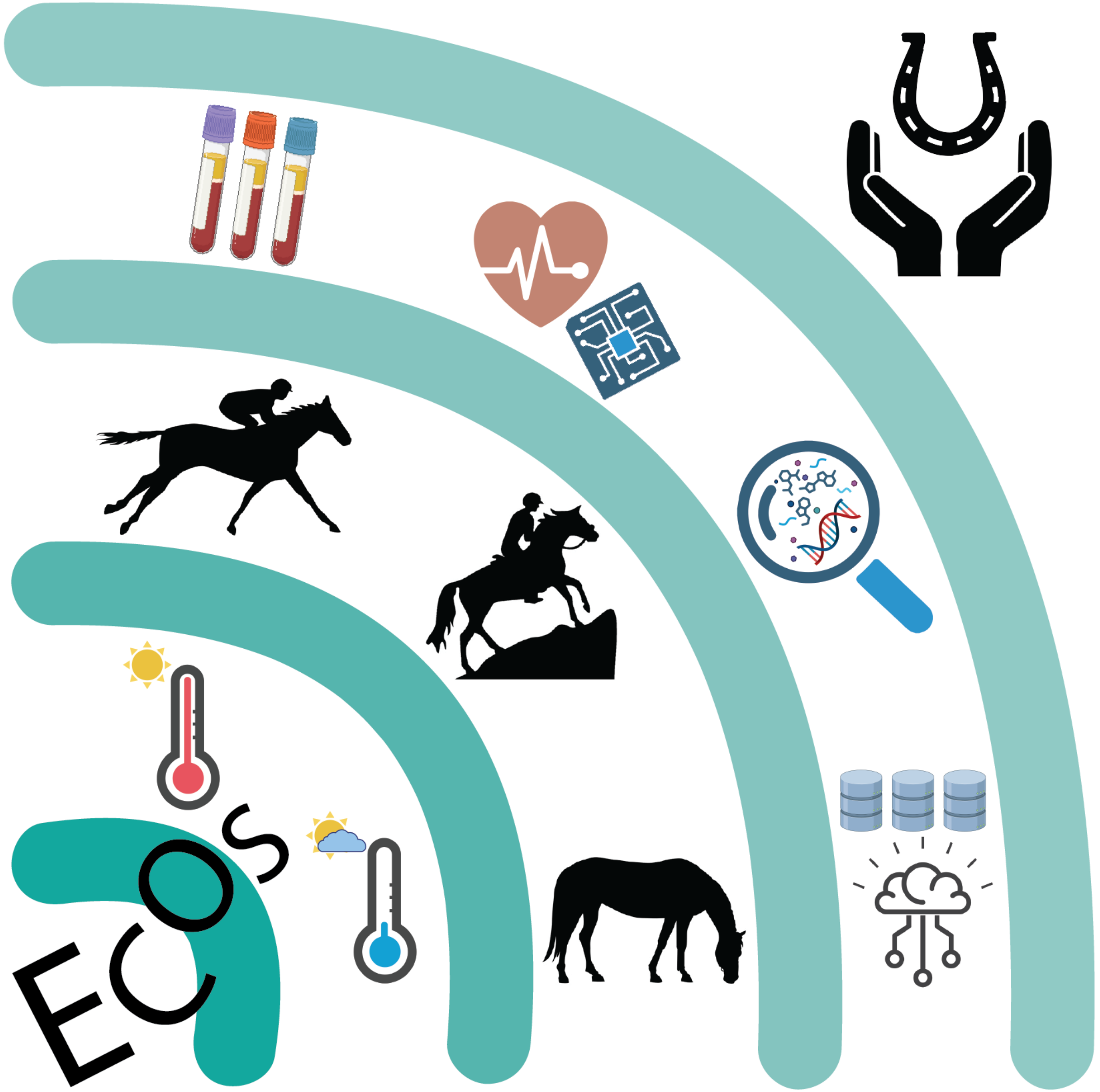
Background: ECOS



Equine resilience and welfare in **C**limate change and stressful scenarios using **O**mic technologies and innovative low cost **S**ensors - ***ECOS***
PRIN2022

ECOS will exploit existing and novel knowledge on horse populations under stress

- ✓ **exercise-induced stress** in athlete horses participating in disciplines that induce the greatest effort
- ✓ **heat Stress** in Southern Italy local breeds to assess response and resilience
- ✓ **combination** of the two stresses in sport horses

For exercise-induced stress **Arabian horses** will be identified

- ✓ high intensity-low duration exercise (sprint gallop)
- ✓ **low intensity-long duration exercise (endurance)**

Background: heat stress in horses

Physical heat transfer in exercising horses: thermal radiation, conduction, convection, and evaporation

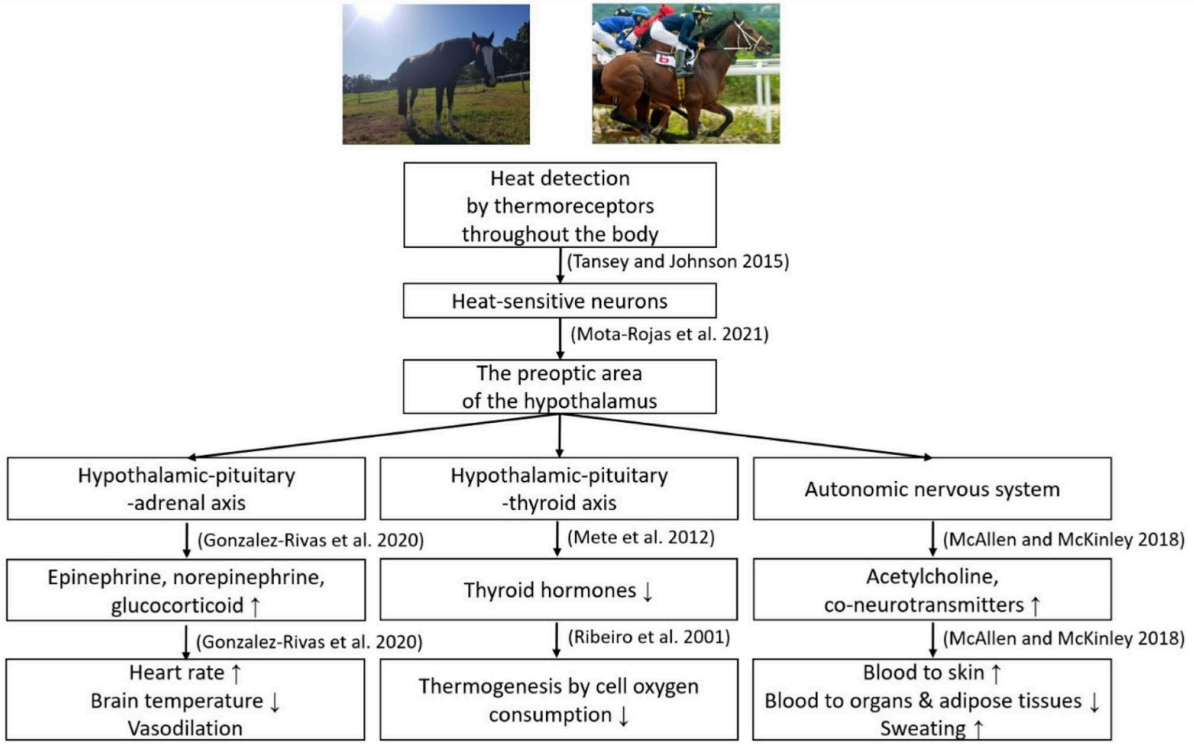


Fig. 2 Neuroendocrine and physiological responses to heat stress. Arrows in the boxes — '↑', increasing; '↓', decreasing. The images were adapted from Hyungsuk Kang and open source 'Adobe stock images'

When heat stress is detected by the horse, the hypothalamic–pituitary–adrenal (HPA) axis and the autonomic nervous system (ANS) are upregulated and hypothalamic-pituitary-thyroid (HPT) axis function is suppressed to maintain body temperature and to prevent excessive heat accumulation

International Journal of Biometeorology (2023) 67:957–973

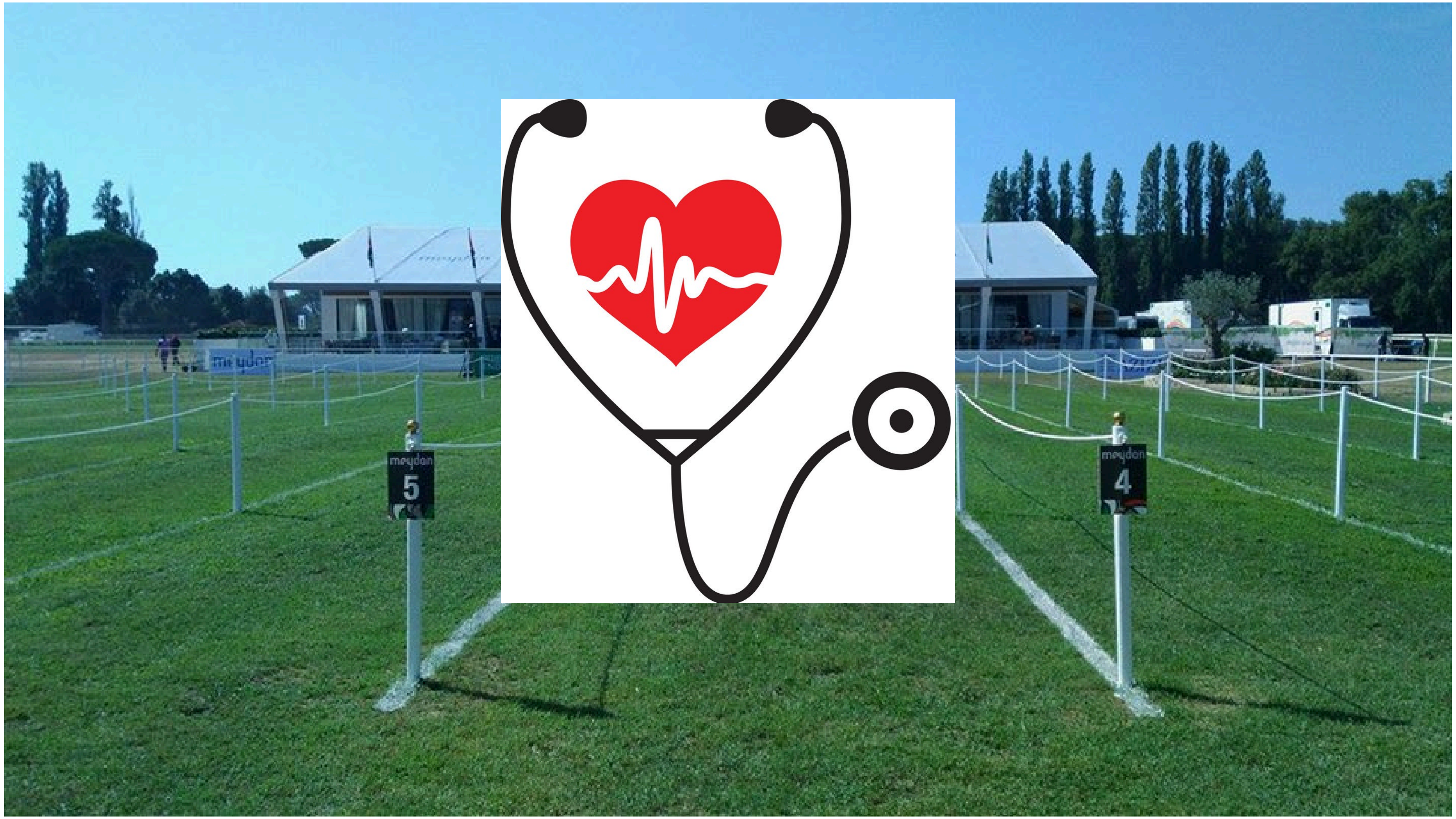
Background: endurance

Endurance race is one of the most challenging discipline due to the long distances (up to 160 km) at an average speed of 20 km/h or more

Endurance horses are susceptible to metabolic imbalance due to dehydration, acid balance and electrolyte abnormalities, substrate depletion and heat accumulation, which can result in life-threatening conditions

Background: endurance

Competitions are governed by the National and International Equestrian Federations (FEI) rules which safeguard and ensure the animal welfare



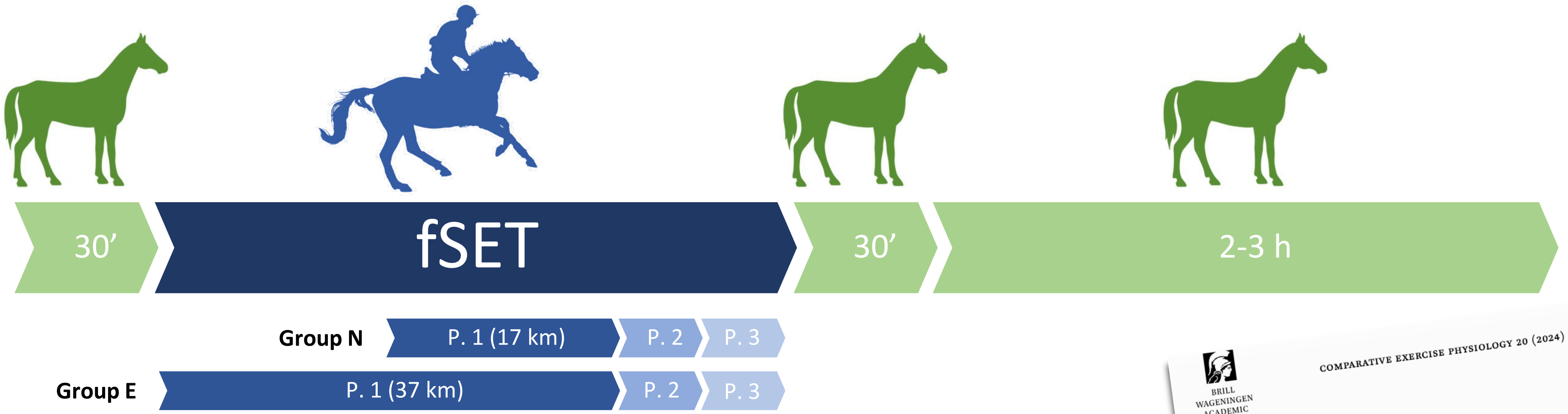
FEI
FÉDÉRATION ÉQUESTRE INTERNATIONALE

FISE

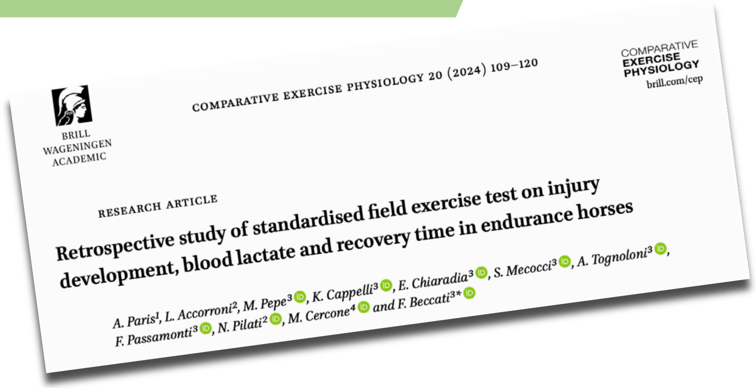
ITALIA
CONI

Background: exercise tests

Incremental field standardized exercise tests (fSETs) are valuable systems for horse training and fitness evaluation (A. Paris et al. 2024)



- 17 or 37 km at 20.8-21.2 km/h
- 1,400 m at 26.8-27.5 km/h
- 1,400 m at 30-32.3 km/ h


COMPARATIVE EXERCISE PHYSIOLOGY 20 (2024) 109–120

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COMPARATIVE EXERCISE PHYSIOLOGY brill.com/cep

RESEARCH ARTICLE

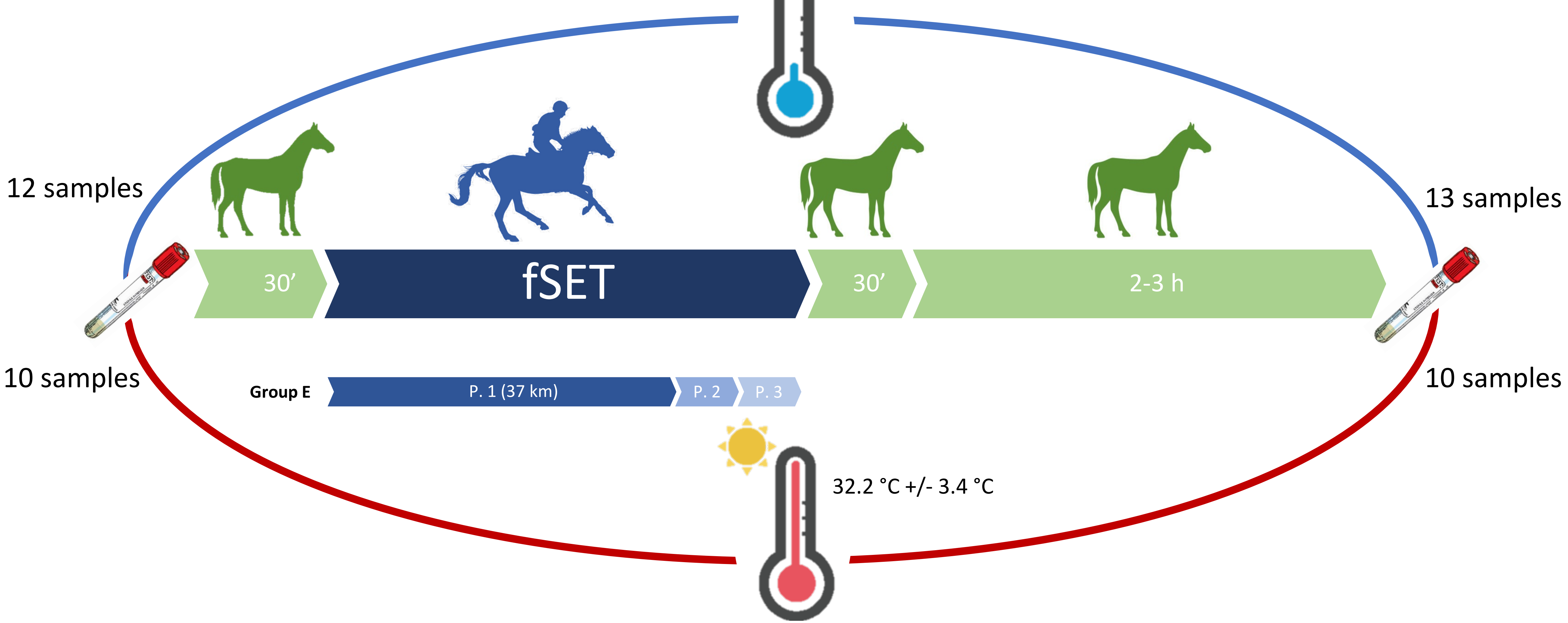
Retrospective study of standardised field exercise test on injury development, blood lactate and recovery time in endurance horses

A. Paris[1], L. Accorroni[2], M. Pepe[3], K. Cappelli[3], E. Chiaradia[3], S. Mecocci[3], A. Tognoloni[3], F. Passamonti[3], N. Pilati[2], M. Cercone[4] and F. Beccati[3]*

Comparative Exercise Physiology 20 (2024) 109–120 ; DOI: 10.1163/17552559-20220059

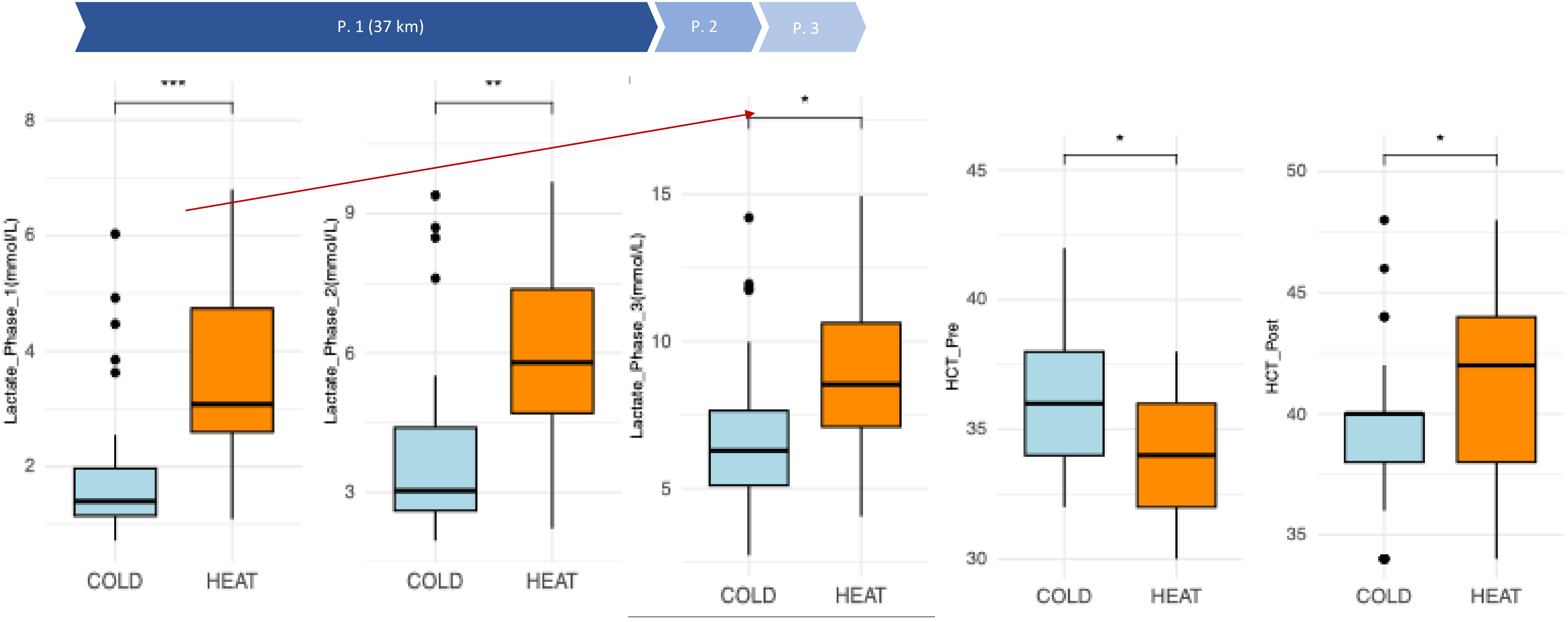
Aim of the study

Experimental schedule and sampling

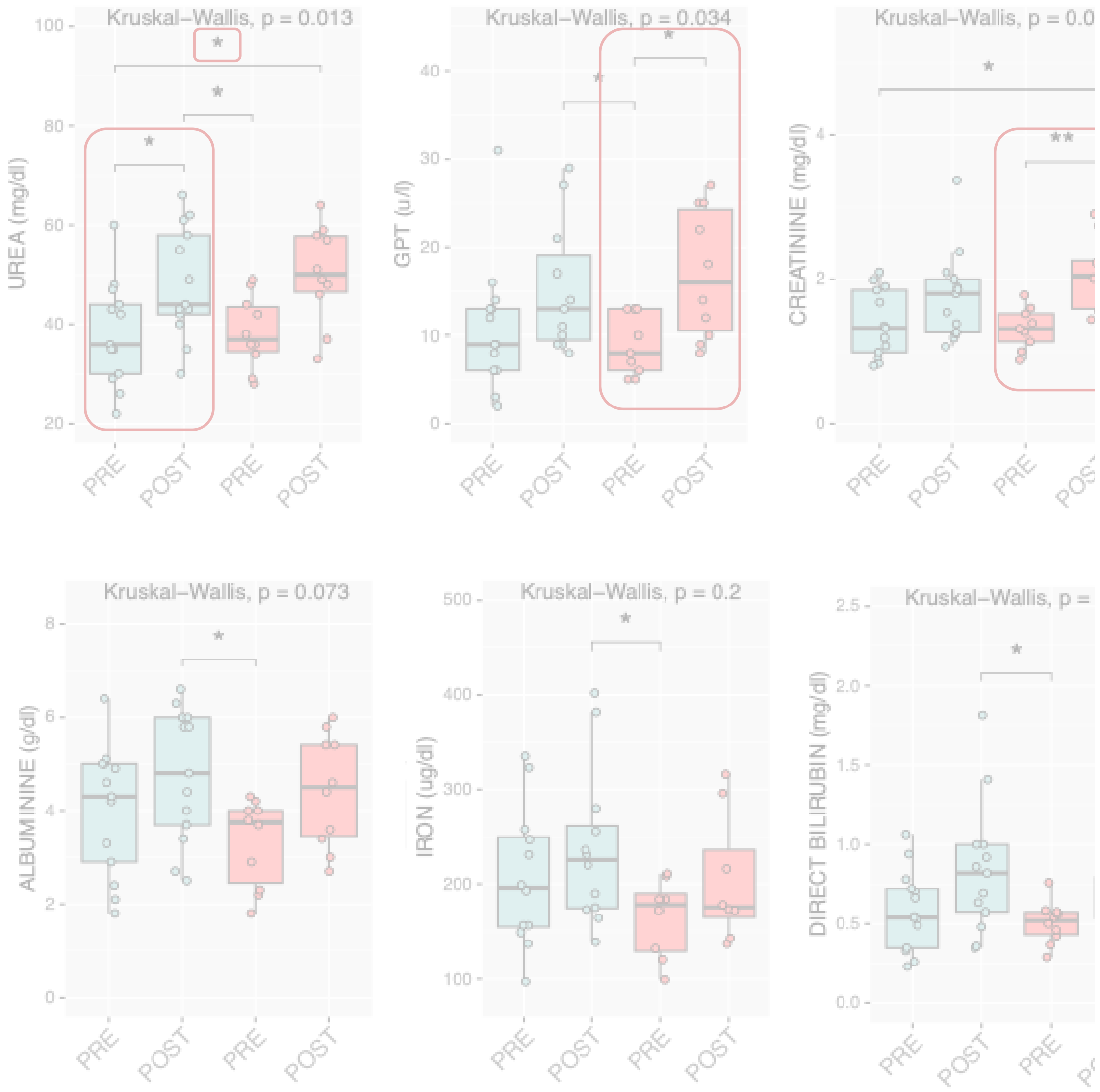


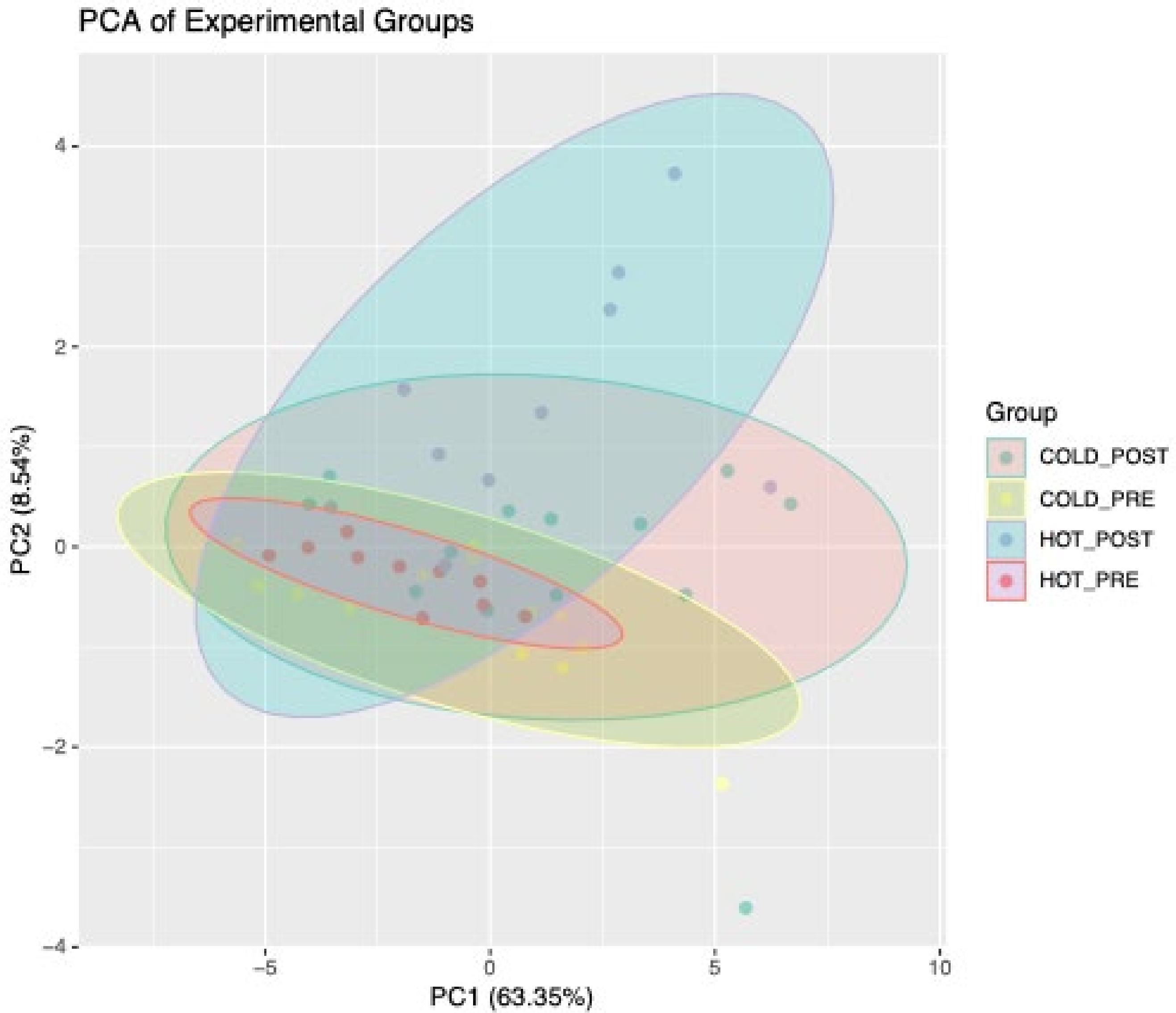
- Clinical biochemist
- Liquid biopsy - Sequencing

Monitoring horse health and performance

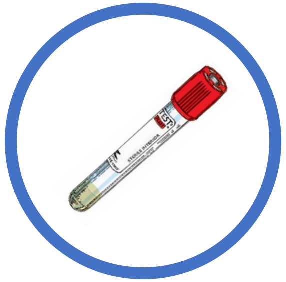


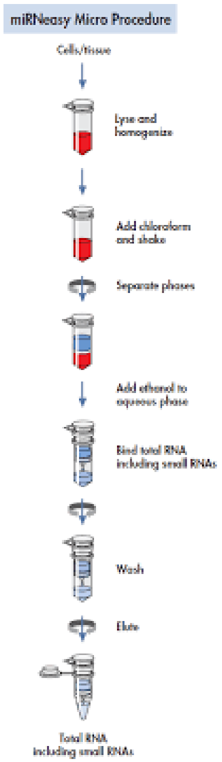
Clinical biochemist results





RNA extraction, sequencing and bioinformatic analysis





Spike-in

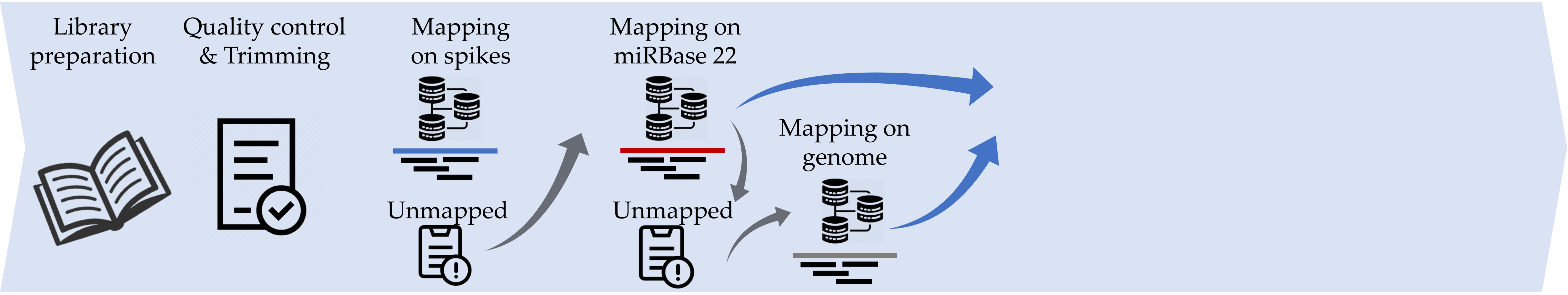
Exogenous RNAs

- QIAseq miRNA library kit
- Aviti (Element Biosciences, San Diego, CA)
- 75bp single-end

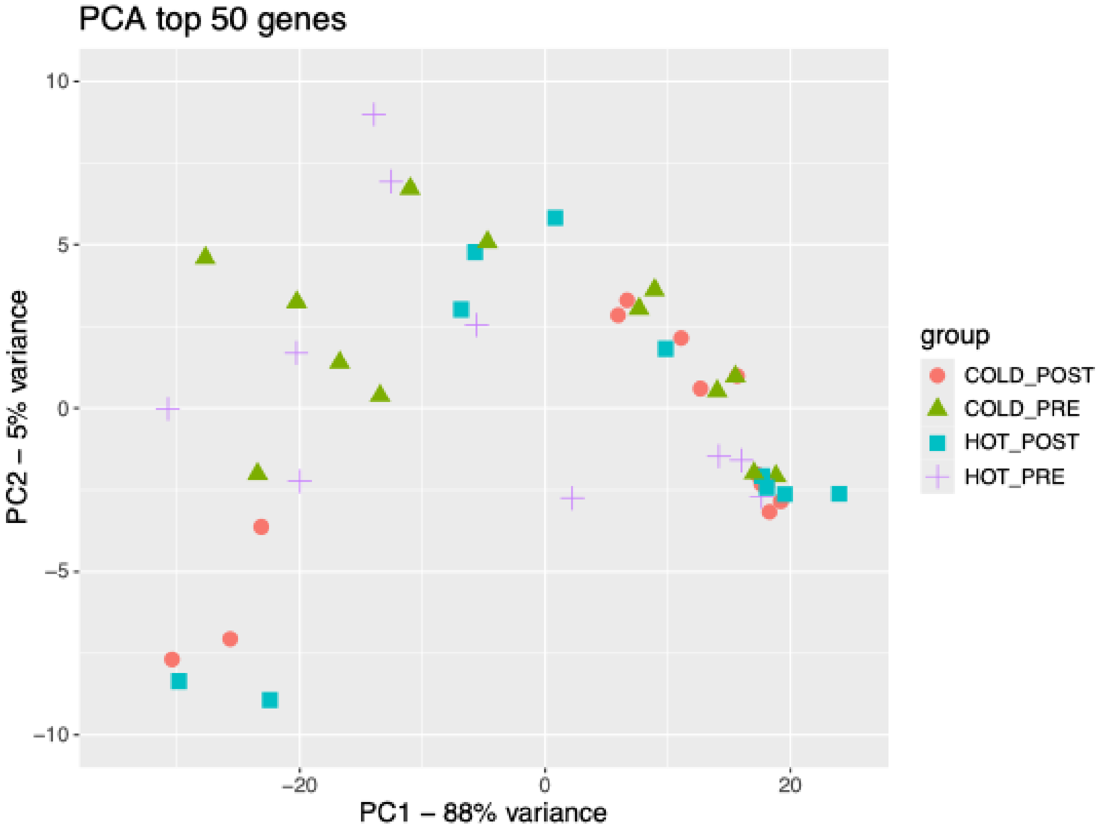
- Over 12M raw reads in everage
- Over 7.7M after trimming

- 15% overall alignment rate of uniquely mapped

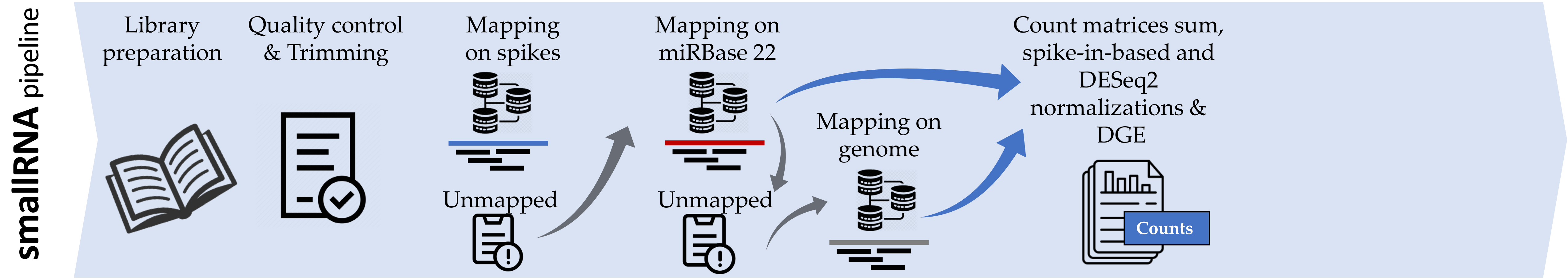
smallRNA pipeline



PCA on NGS data



Similar to the PCA of the hematobiochemical results

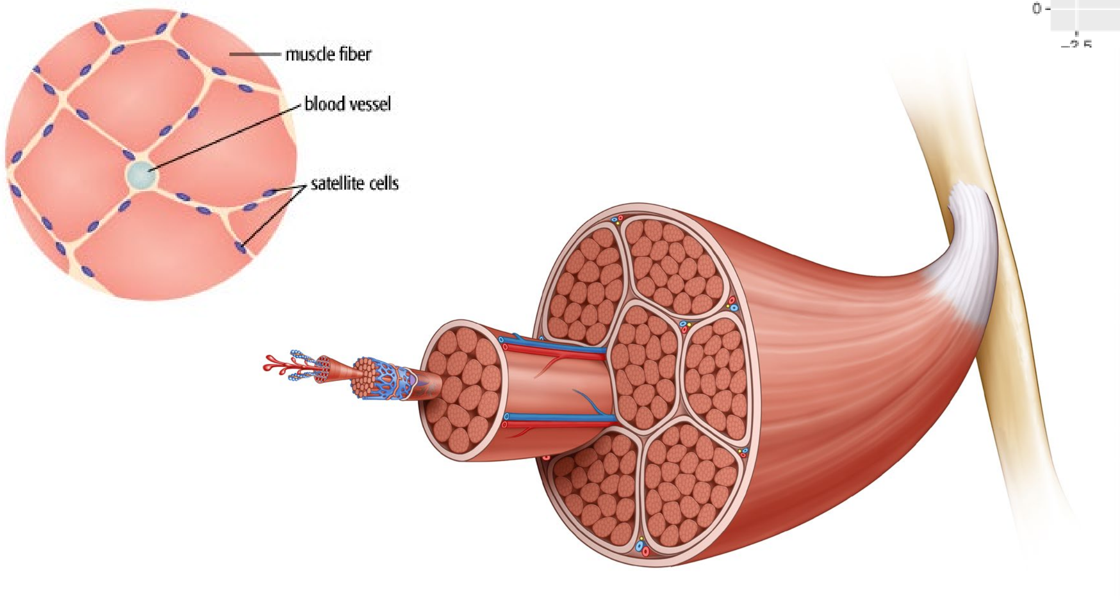


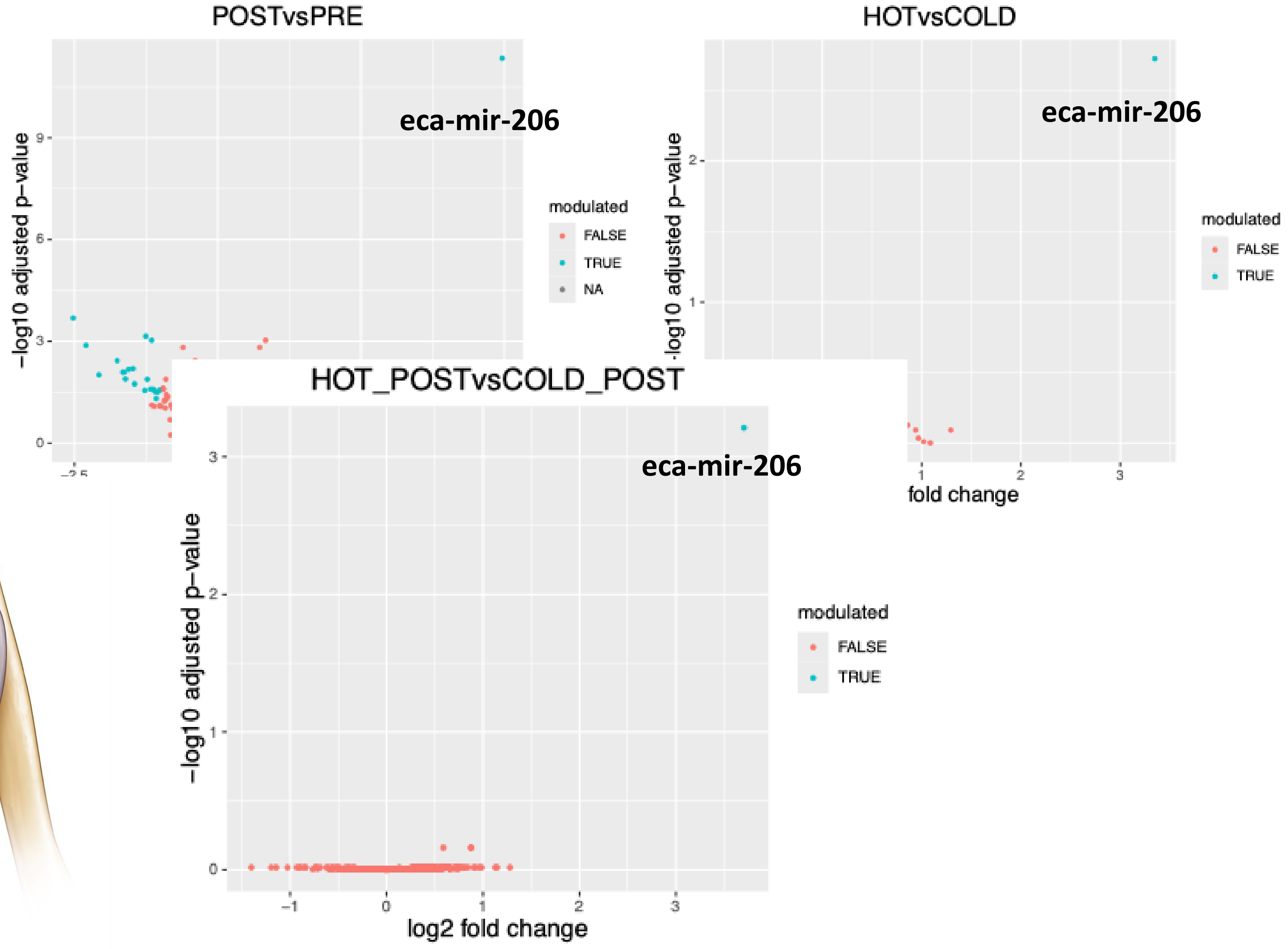
DE miRNAs

MYOMIR

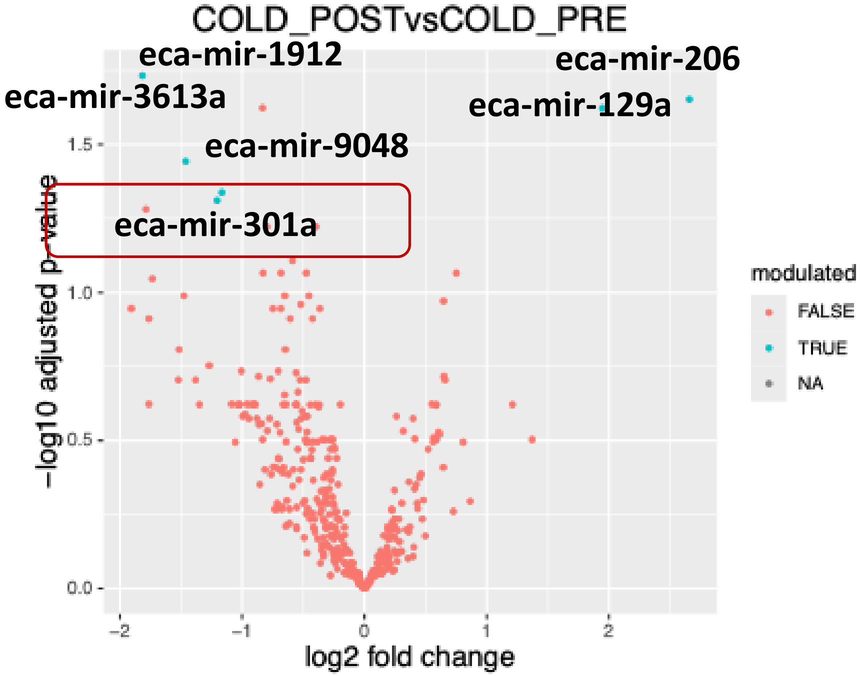
central to the development and health of mammalian skeletal and cardiac muscles

regulation of myogenesis, muscle development and muscle remodeling



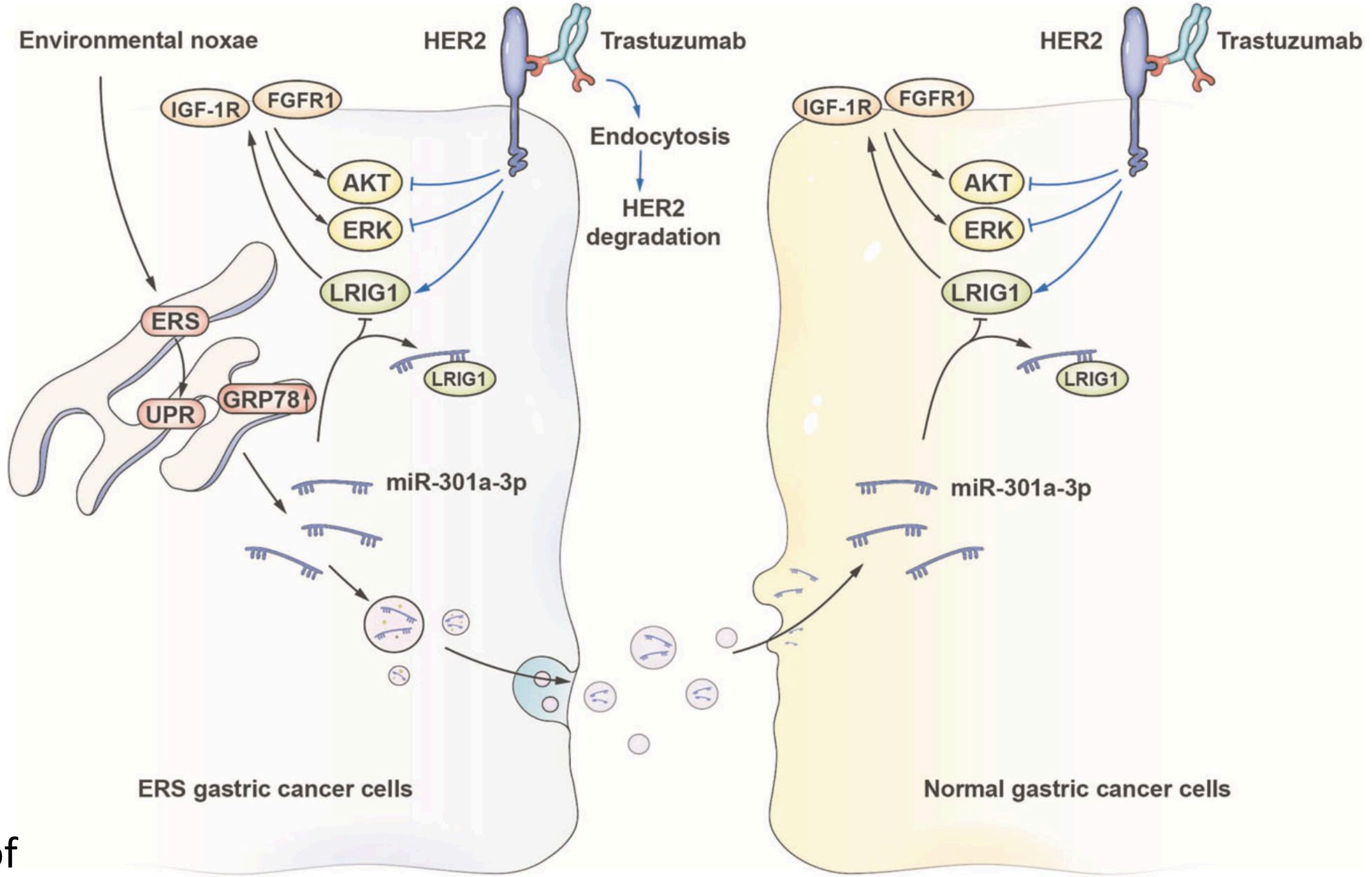


DE miRNAs



mir-301 is released by the cells through exosomes in stress conditions of endoplasmatic reticulum

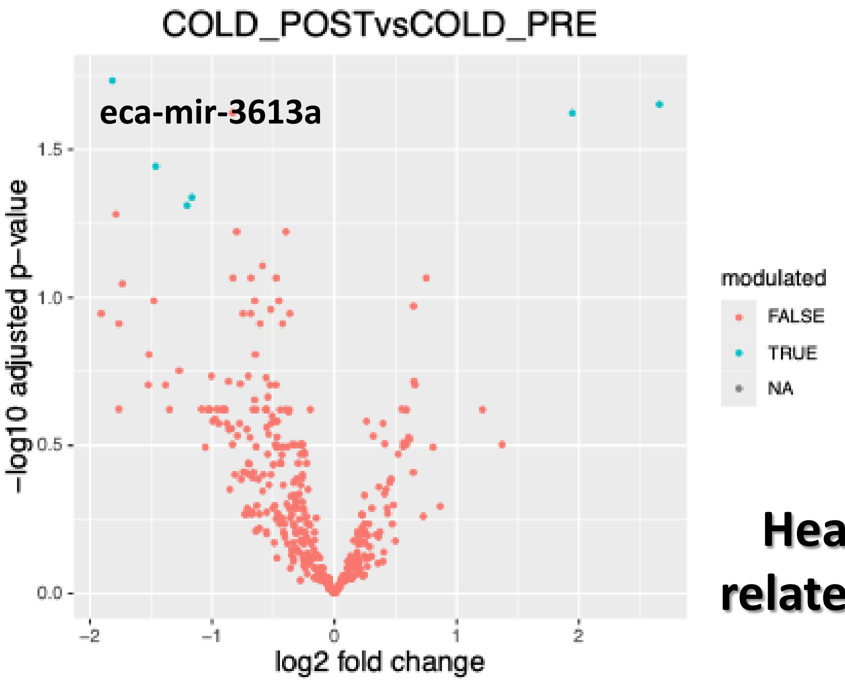
One of its target is LRIG1, an important negative regulator of receptor protein tyrosine kinase (RTK). The LRIG1 silencong by mir-301 increases the levels of IGF-1R and FGFR1



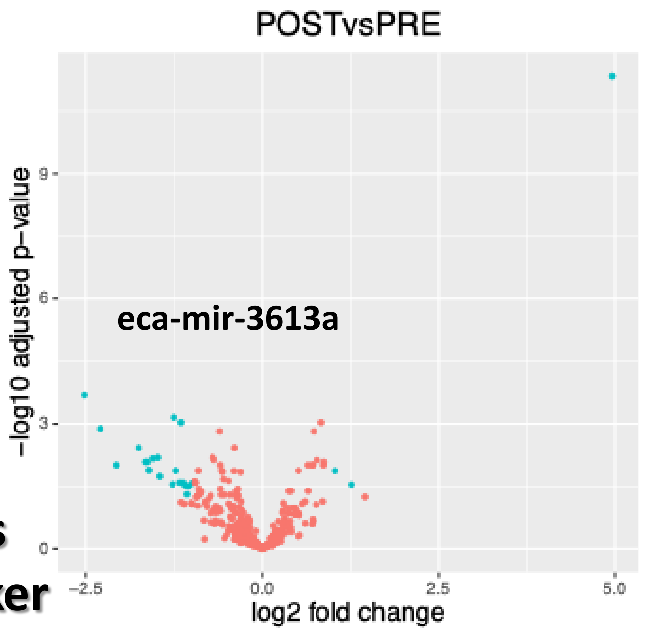
mir-301 induces oxidative stress and apoptosis throught the targeting KFL7

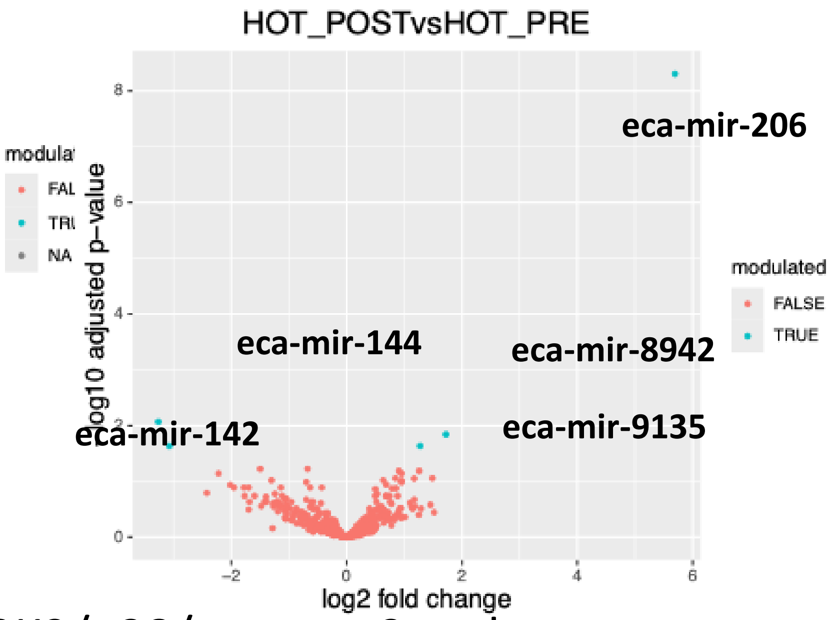
Cell Death and Disease (2021)12:696 ; DOI: 10.1038/s41419-021-03991-3

DE miRNAs



Heat stress related marker





<u>Heat stress suppressed miR-3613-3p</u> expression, which activated the MAP3K2/p38/caspase-3 pathway, leading to the cell apoptosis.

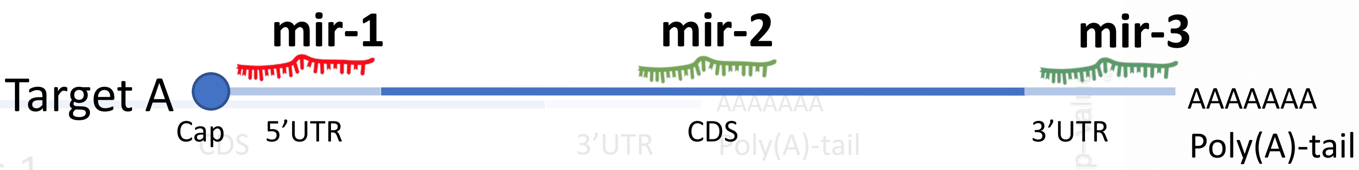
Regulates the expression of the heat shock protein HSPA1A

HS can activate different MAPK signalling pathways in a variety of cell types.

Role of miR-3613-3p <u>during heat stroke</u>, suggesting the modulation of miR-3613-3p as a potential therapeutic strategy for heat stroke.

Target retrieving huge complexity

Target selection

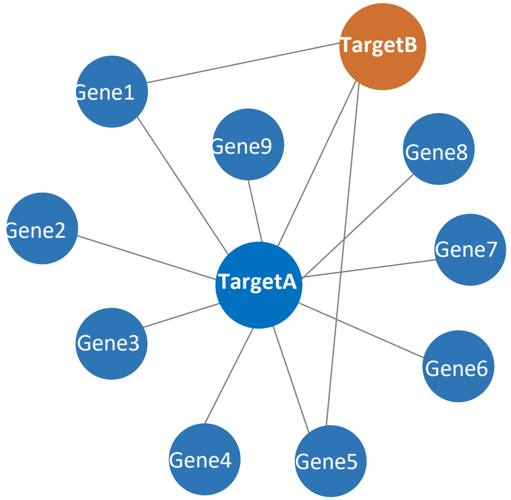


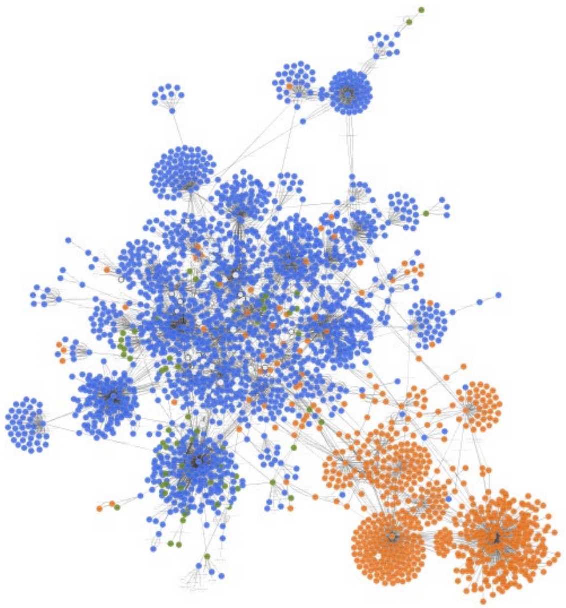
Targets were filtered basing on a threshold of miRNA hits
50% of miRNAs

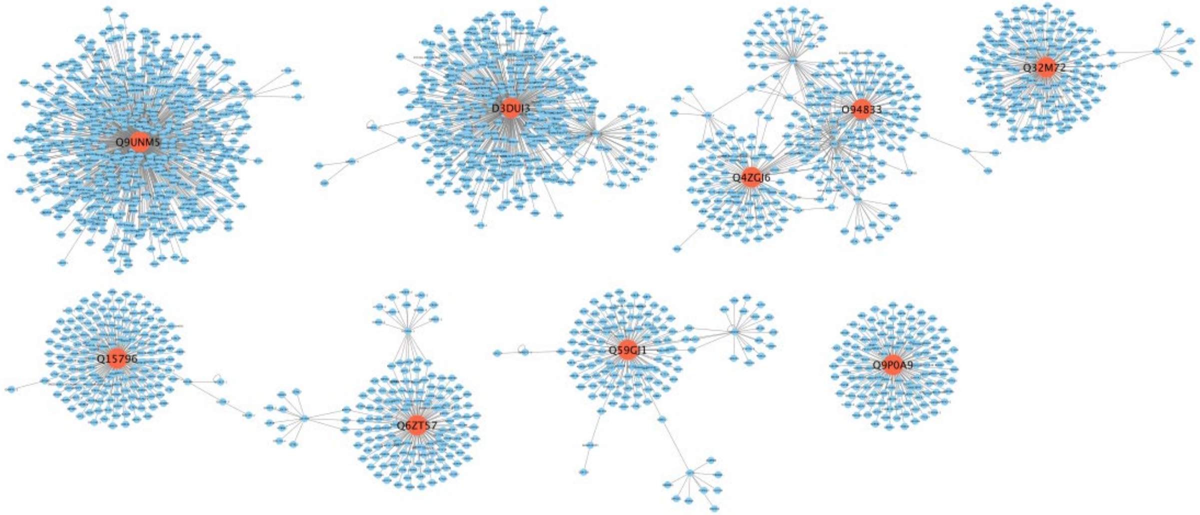
PPI-network construction

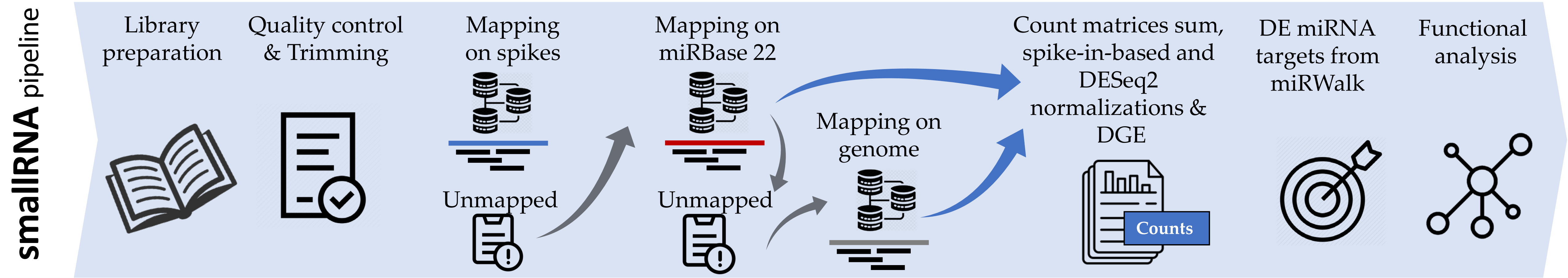
Protein-protein interaction clusters and networks were created starting from the selected targets

IMEx Cytoscape





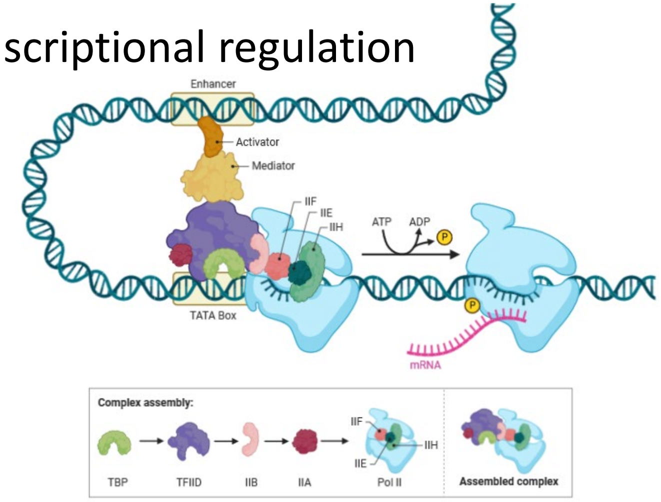


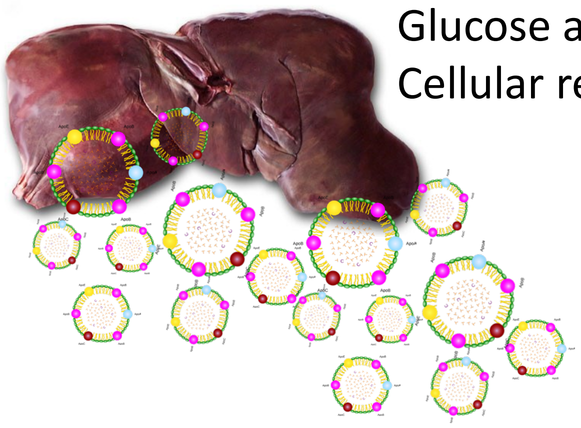


POST vs PRE functional analysis

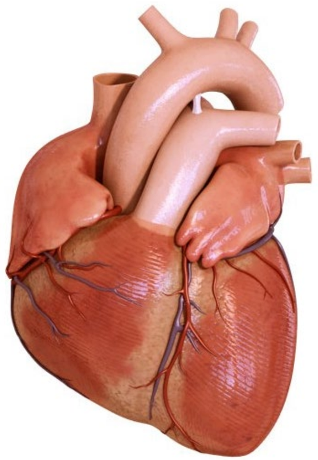
23 modulated miRNAs, most of them (20) were down-regulated

Transcriptional regulation

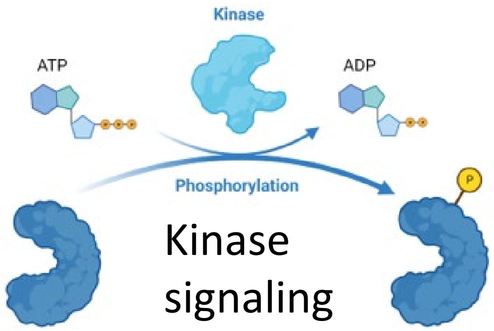




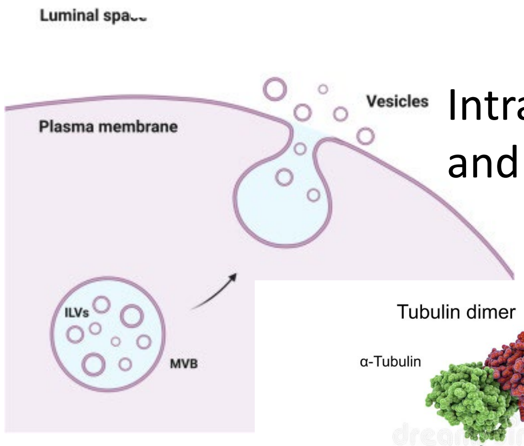
Glucose and LDL response
Cellular response to insulin stimulus



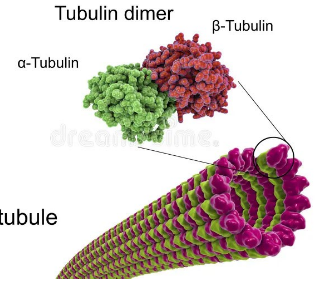
Cardiac conduction



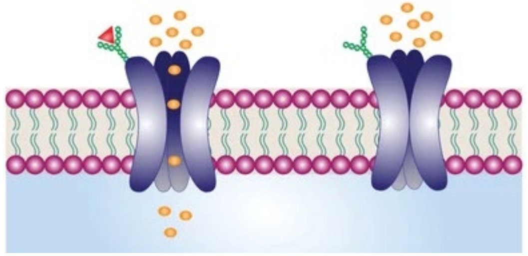
Kinase signaling pathways

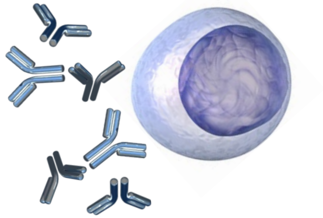


Intracellular-intercellular trafficking and cytoskeleton organization



Ion channels

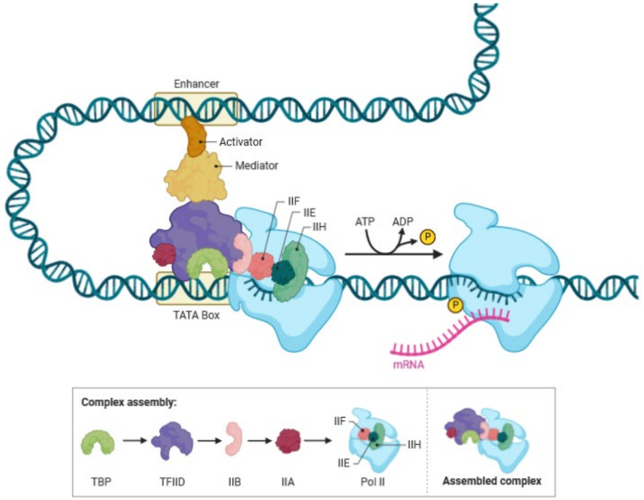




Immune regulation
Regulation of INF type 1
Regulation of TGFB receptor signaling pathway

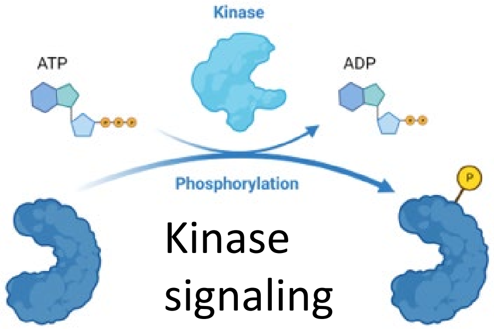
HOT_POST vs HOT_PRE functional analysis

Focusing on down-regulated miRNAs in the hot period: eca-mir-142 and eca-mir-144

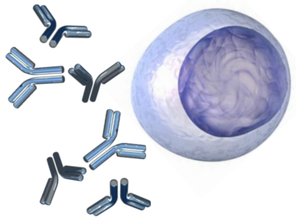


Regulation of transcription in response to stress

Negative regulation of STAT phosphorylation



Kinase signaling pathways



Positive regulation of dendritic cell cytokine production
Negative regulation of inflammatory response to antigenic stimulus
T cell co-stimulation
Negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathways
Apoptotic signaling

Conclusions

Differences in the circulating transcriptome between the two comparisons
- pre-post training and hot and cold season -
early triggering of homeostasis restoration systems

No serious signs of decompensation were highlighted from both hematobiochemical and ci-miRNA data

The results show that this group of athletes is well trained and adapted to stressful conditions due to training even in the hot season

Confirmation of ci-miRNAs as potential biomarker molecules of the organism's response to conditions of heat and exercise stress

The identified miRNAs and their target genes could be useful to understand the mechanism of successful adaptation to stress and to plan training

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Stefania Bergagna

Thank you for your attention!

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