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PROJECT FINAL REPORT

Grant Agreement number: 277936

Project acronym: META-PREDICT

Project title: Developing predictors of the health benefits of exercise for individuals

Funding Scheme: [HEALTH.2011.2.4.3-3 HEALTH.2011.2.4.2-2 HEALTH] Molecular and physiological effects of lifestyle factors on diabetes/obesity. Evaluation and validation studies of clinically useful biomarkers in prevention and management of cardiovascular disease Health.

Period covered: from 01/12/2011 to 01/07/2016 (incl. extension 6 months)

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4.1 Final publishable summary report

THIS FILE CONTAINS THE FIGURES AND TABLES ONLY. These belong to the text submitted online

4.1a - Executive Summary

4.1b - A summary description of project context and objectives

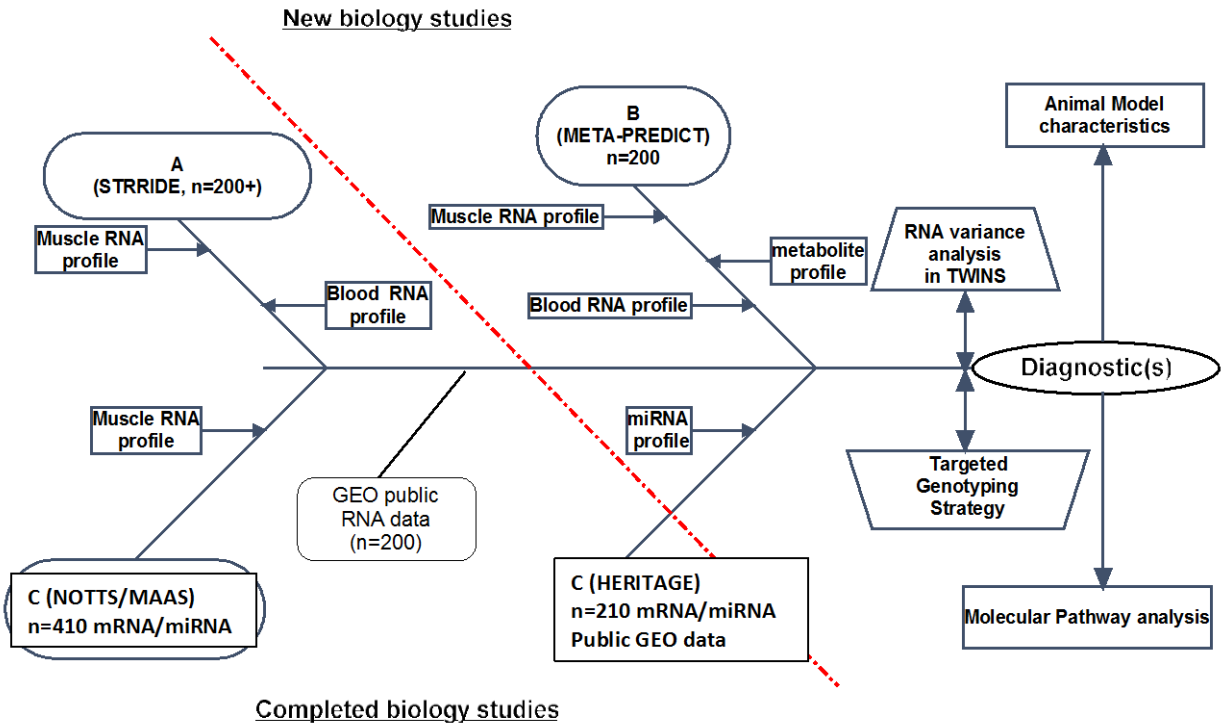


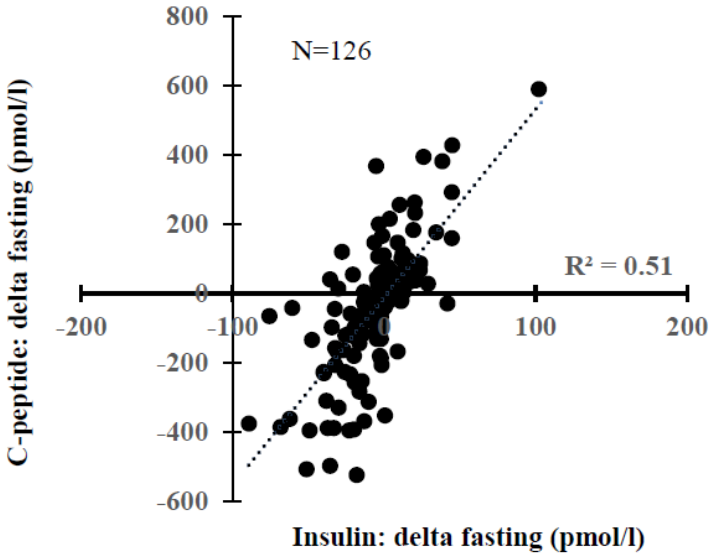
Figure 1. A high-level overview of the META-PREDICT*. We have independent exercise life-style modification programs (**A-D**) with common clinical end-points that feed into a 'predictor' pipeline where we generate diagnostic screens from RNA, mDNA and metabolites. This will lead to personalised diagnostics, greater understanding of the underlying biology, and biomarkers for validating a novel drug screening in vivo model. Studies A and part of D have existing RNA gene-chip profiles; Study C has miRNA and mRNA deposited in the public domain (GEO). Study B will be the largest study of time-efficient cycle-sprint training in at-risk (metabolic disease) subjects studied using HTA 2.0 RNA, miRNA and mDNA chips.*

4.1c - A description of the main S&T results/foreground

Figure 2. Metabolomics analyses in a smaller cohort of Twins *with significant difference in level of physical activity (left) which was confirmed in larger cohorts of matched individuals with high and low levels of physical activity. From original publication (16).*

Table 1. Interval-training induced concentration differences between HRT and LRT female rats. *The concentrations for the metabolites are given as mean*±SD in *μM.TRP:tryptophane.*

Metabolite (µM)	Female HRT		Female LRT		p-value	FDR
	PRE	POST	PRE	POST		
Lauroylcarnitine	0.04±0.02	0.02±0.01	0.03±0.02	0.02±0.01	*3.97E-09*	*1.71E-07*
Stearoylcarnitine	0.11±0.10	0.05±0.04	0.07±0.04	0.04±0.02	*5.30E-08*	*1.14E-06*
Octanoylcarnitine	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.02	*1.23E-07*	*1.77E-06*
Myristoylcarnitine	0.12±0.09	0.04±0.03	0.10±0.07	0.04±0.02	*7.69E-07*	*8.26E-06*
Hexanoylcarnitine	0.05±0.02	0.05±0.02	0.05±0.03	0.09±0.06	*1.27E-05*	*0.0001094*
TRP	107.4±9.7	98.8±9.5	102.5±15.1	98.4±13.1	*0.0016521*	*0.01184*



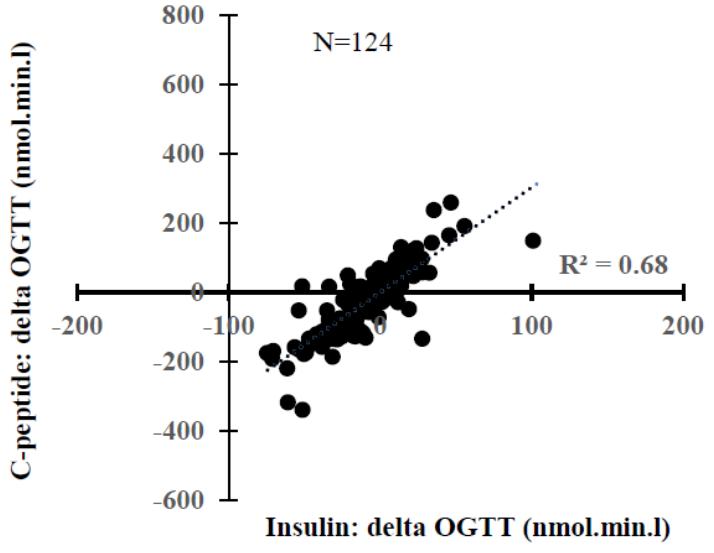


***Figure 3:* Human Studies** *- Presentation of the linear relationship between changes in plasma insulin and plasma C-peptide concentrations following 6 weeks of '5-by-1' HIT protocol. Oral glucose tolerance test (OGTT). Value is based on Pearson correlation coefficients (coefficient of determination).*

Table 2. Subject characteristics for the HIT study

	Non-exercise (n=13)	**'7-by-1' HIT** (n=40)	**'5-by-1' HIT** (n=137)
Gender (men/women)	4 / 9	20 / 20	63 / 74
Age (y)	25 (20-51)	38 (20-53)	38 (18-51)
Height (m)	1.66 (1.52-1.81)	1.71 (1.53-1.94)	1.72 (1.51-2.01)
BMI ($kg \cdot m^{-2}$)	32.7 (27.3-41.4)	29.8 (27.0-45.5)	31.2 (26.5-48.1)
Fasting glucose ($mmol \cdot L^{-1}$)	4.6 (4.0-5.2)	4.5 (3.8-5.4)	4.6 (3.3-5.6)
2hr OGTT glucose ($mmol \cdot L^{-1}$)	6.3 (5.2-9.4)	6.5 (4.2-10.5)	6.8 (3.7-10.1)
IPAQ Score	248 (160-1188)	382 (73-594)	330 (0-597)

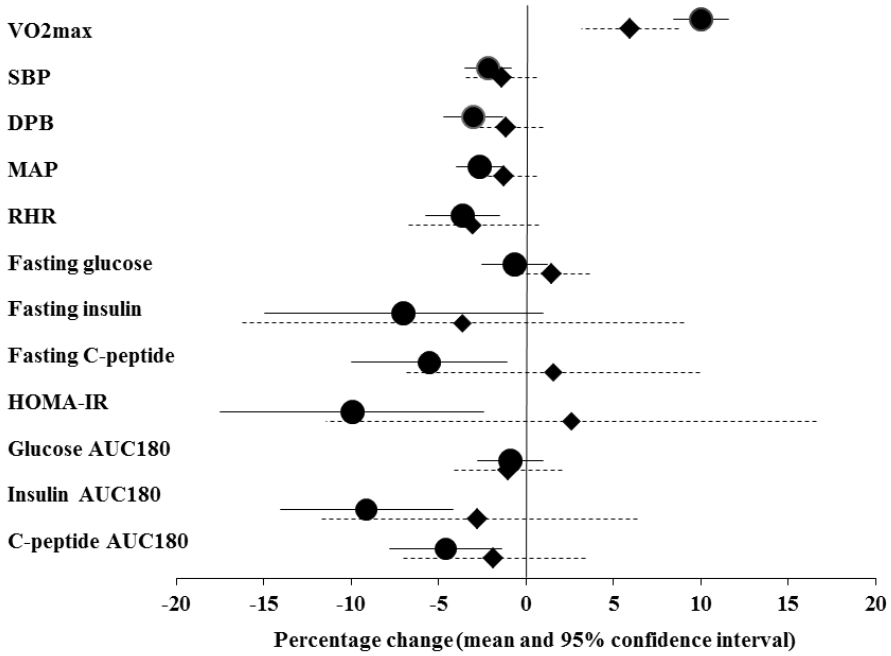


Figure 4: Comparison of the efficacy of 6 weeks of the '5-by-1' HIT high intensity cycle based exercise training protocol (black circles) with the less effective '7-by-1' HIT (black diamonds) protocol in obese or over-weight adults. *The values are presented as mean ± 95% CI for the main training responses. AUC: area under the curve during oral glucose tolerance testing (180 min); SBP: Supine systolic blood pressure; DBP: Supine diastolic blood pressure; MAP: Supine mean arterial pressure; RHR: resting heart rate.*

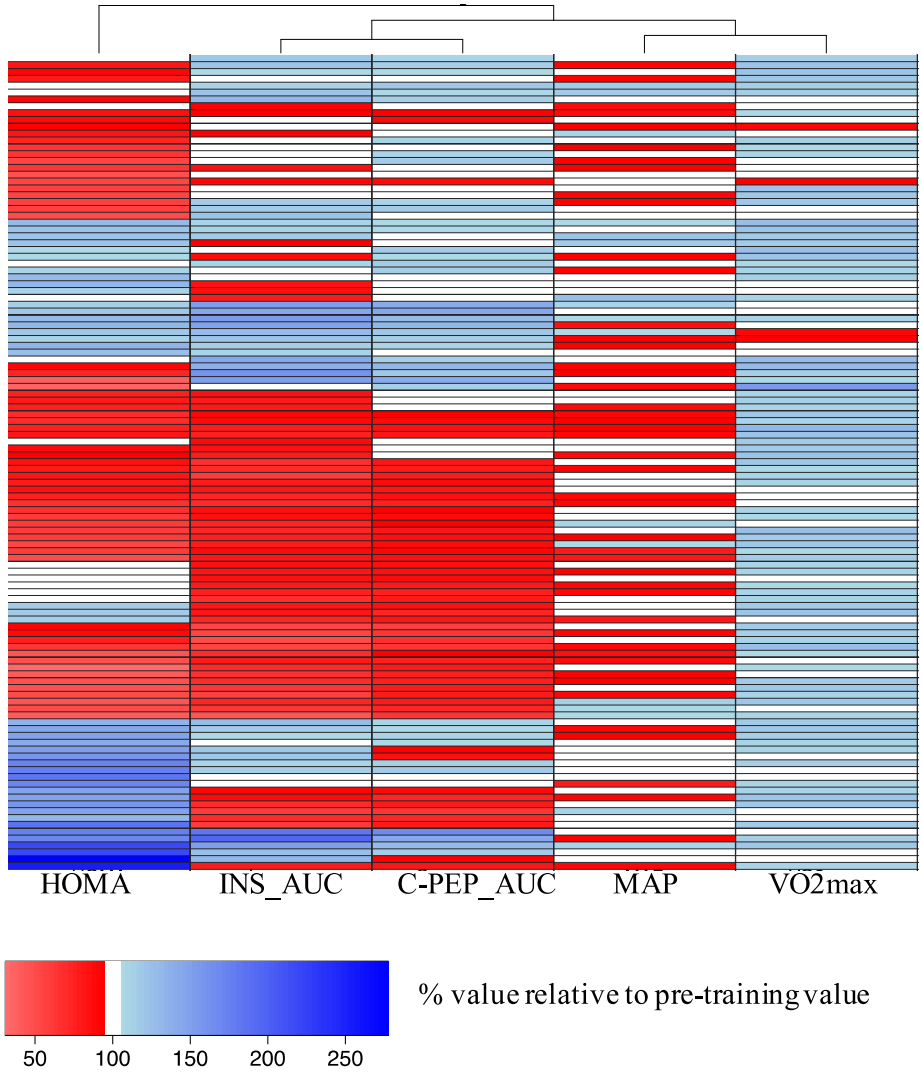


Figure 5: Heat-map representation of the individual responses to exercise training for 5 variables following '5-by-1' HIT *($\dot{V}O_2$max, MAP, Oral glucose tolerance test (OGTT) Insulin AUC_{180} and HOMA-IR). It can be observed that each subject demonstrates a differential response to the 5 clinical parameters. Some subjects improve all five, most improve 2 or 3 while some do not improve any of these health biomarkers.*

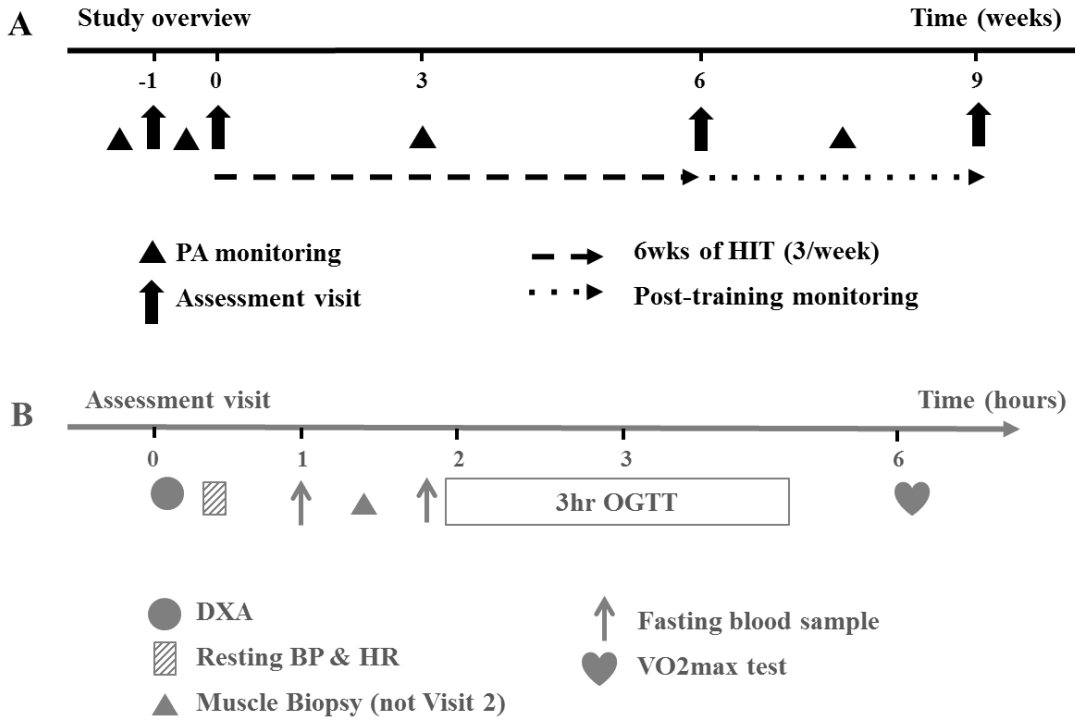


Figure 6. META-PREDICT HIT study design. *Six weeks of supervised HIT was preceded by two study visits (baseline measurements, one week apart) and followed by another two study visits, post-training (~3 days after final session) and post-training monitoring (3 weeks after the final session). After an overnight fast, a DXA scan was followed by two fasted blood samples and a muscle biopsy, a standard 75-g OGTT, a standardised meal and a $\dot{V}O_2$max-test. The DXA scan and muscle biopsies were not performed during Study visit 2.*

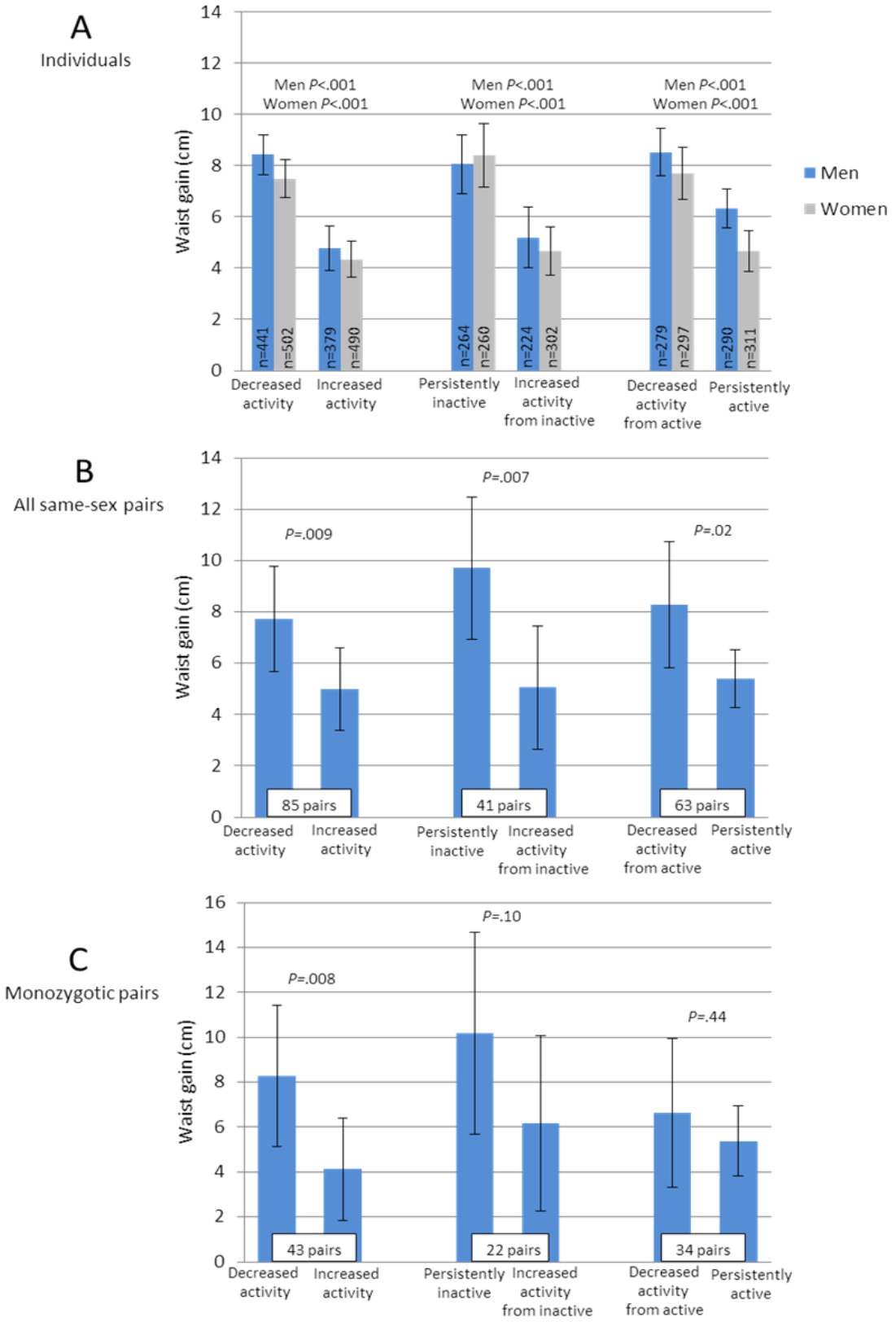


Figure 7. Differences in waist gain (cm, mean and 95%CI) during follow-up (decreased activity: changed from upper third to a lower one; increased activity: changed from a lower third to upper one). *(A) Sex-specific differences among individuals taking into account clustered observation of twin pairs. (B) Pairwise difference among leisure-time physical activity discordant same-sex twin pairs. (C) Pairwise differences among leisure-time physical activity discordant monozygotic twin pairs (from (28)).*

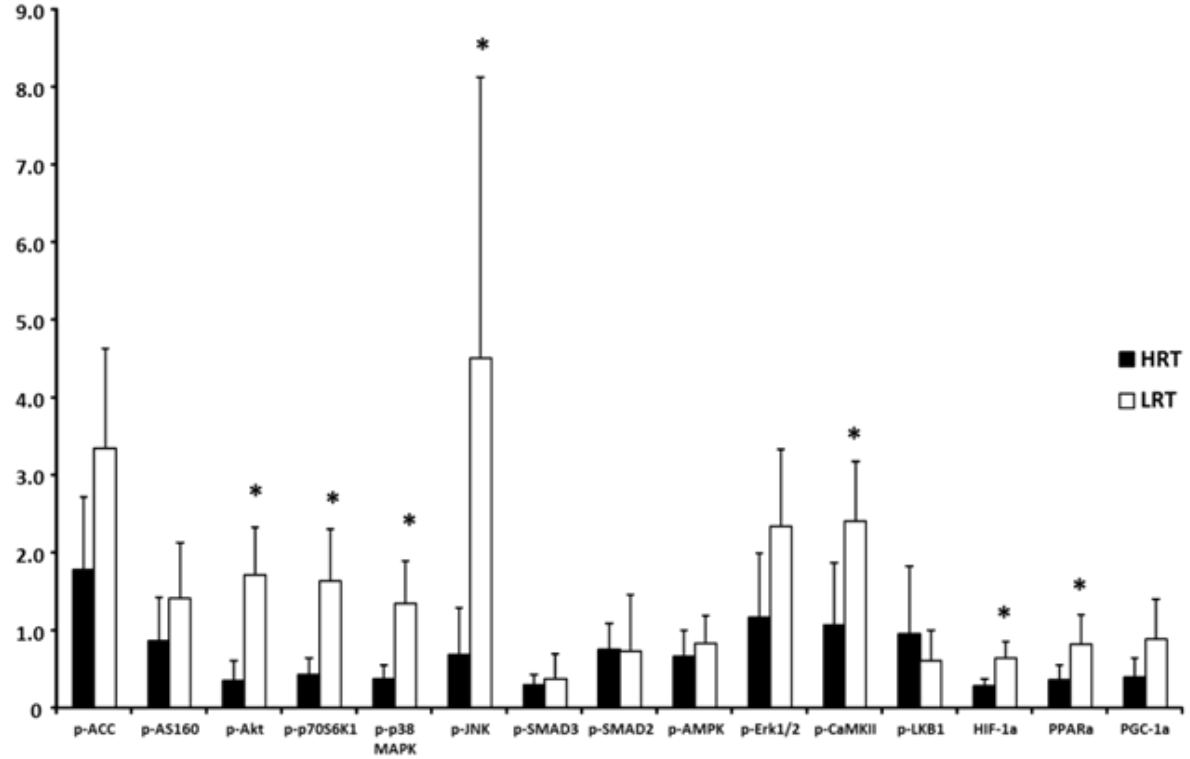


Figure 8. Content of selected signaling proteins *[mean (SD)] in skeletal muscle (m.gastrocnemius) of high and low responder rats following acute high intensity exercise. * = statistically significant difference between the rat lines.*

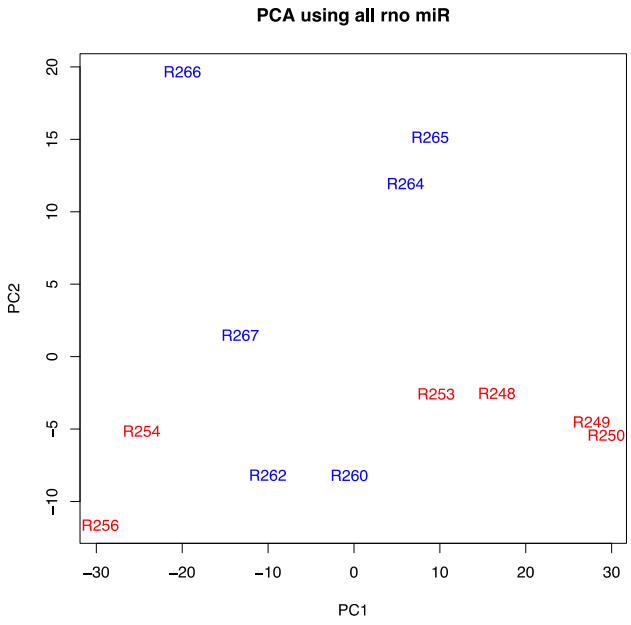


Figure 9. The impact WP5 D5.3 experiment on Rat miRNA PCA *(Affymetrix)*

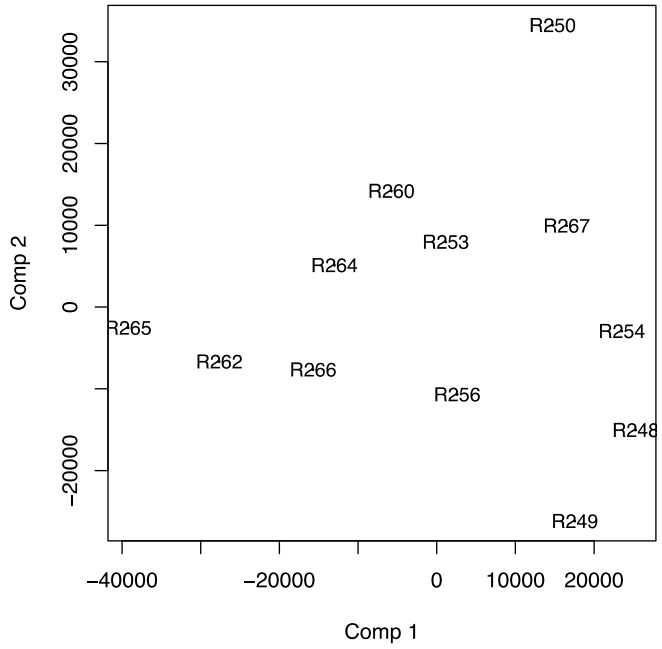


Figure 10. The impact WP5 D5.3 experiment on Rat miRNA PCA (Exiqon)

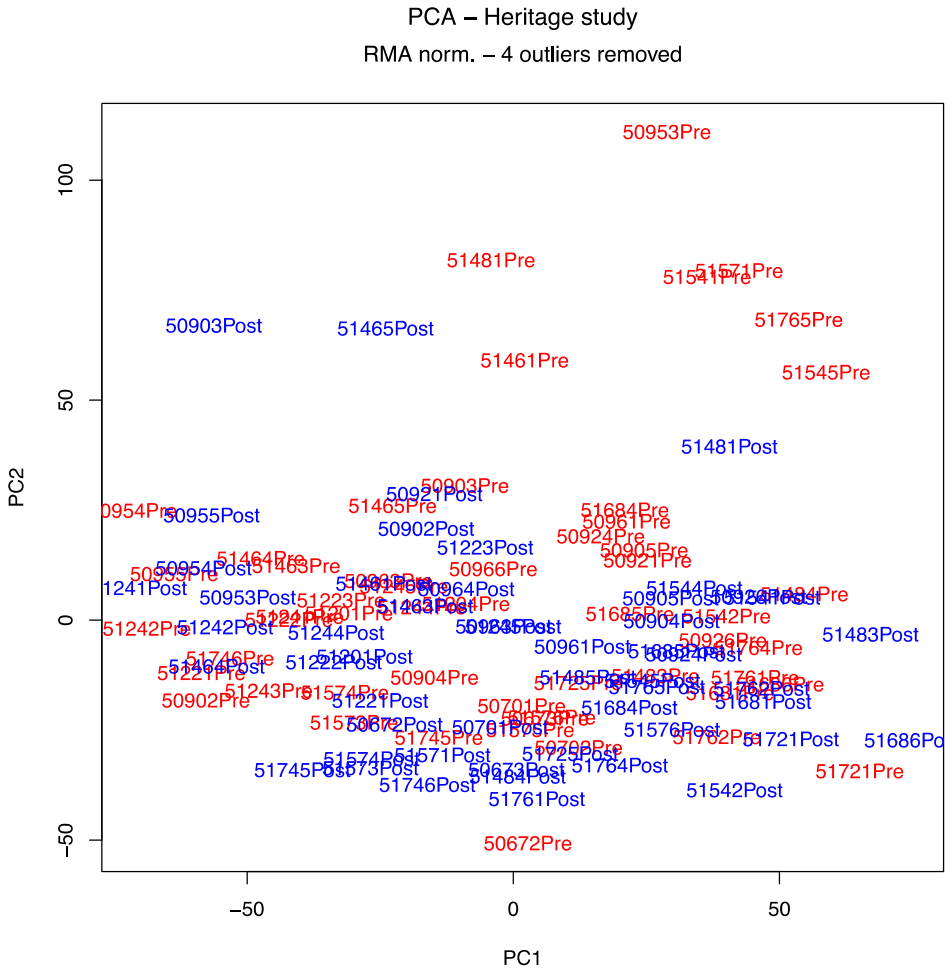
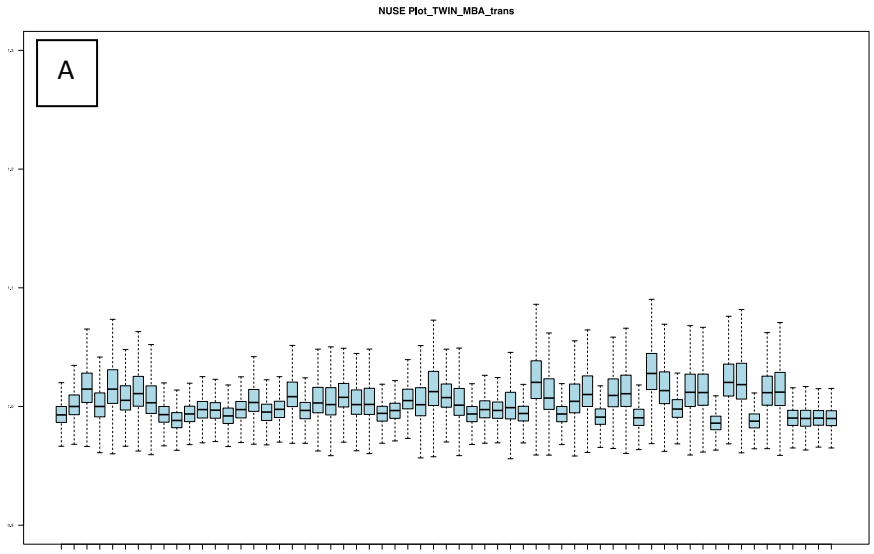
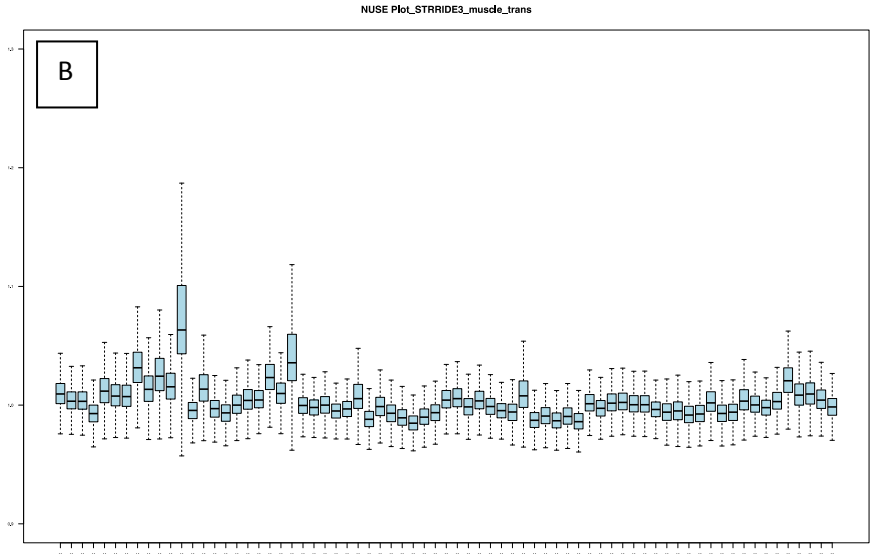
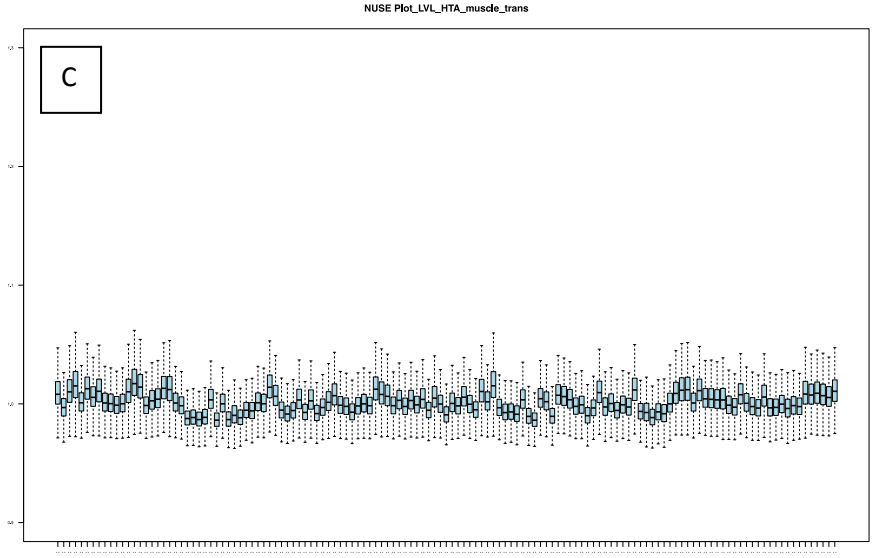


Figure 11 - Affymetrix miRNA array 2.0 PCA plot – Heritage Samples


NUSE Plot_TWIN_MBA_trans
A


NUSE Plot_STRRIDE3_muscle_trans
B


NUSE Plot_LVL_HTA_muscle_trans
C

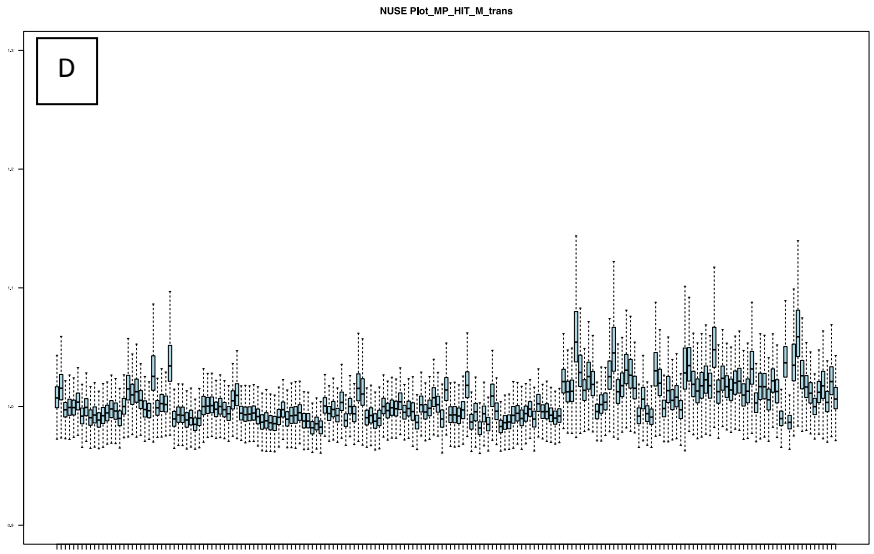


Figure 12. NUSE plots for muscle samples *from A) the TWINN study, B) STRIDDE III, C) LVL Maastricht study and D) HIT study.*

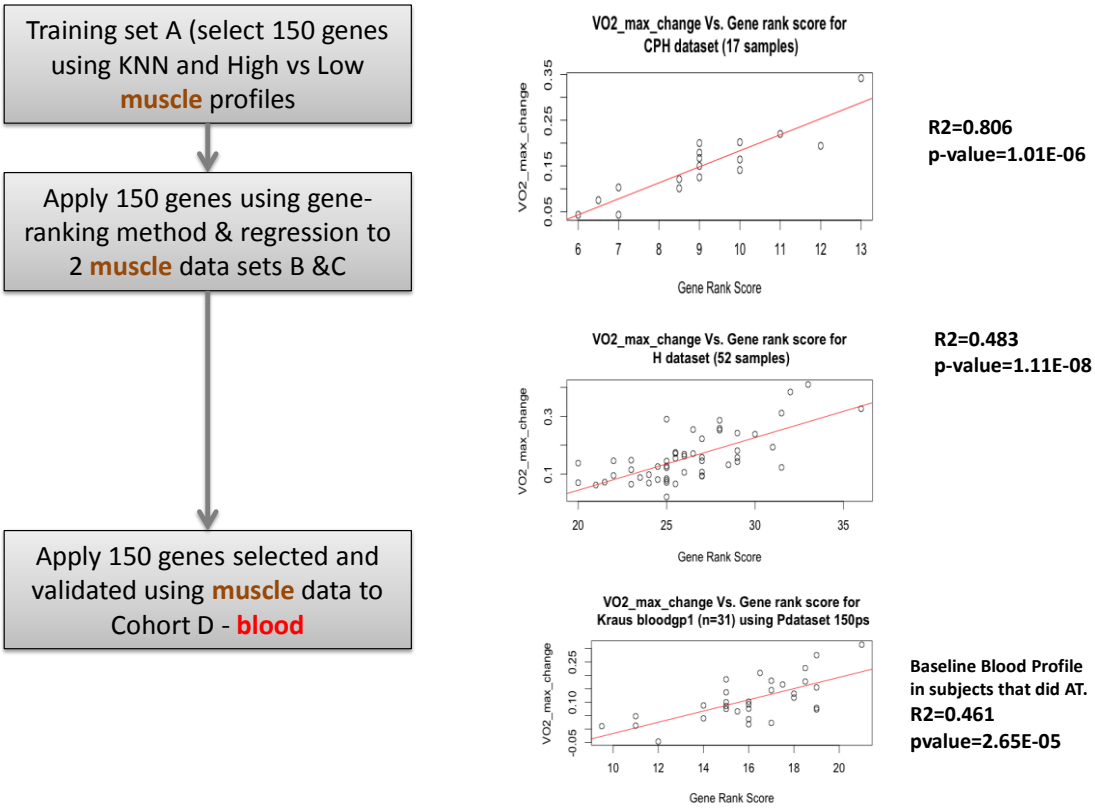


Figure 13*. Using new (WP8) DUKE blood chip data (n=124 baseline from 289) we took the muscle based aerobic predictor signature genes and attempt to reproduce this classifier from the blood RNA profile.*

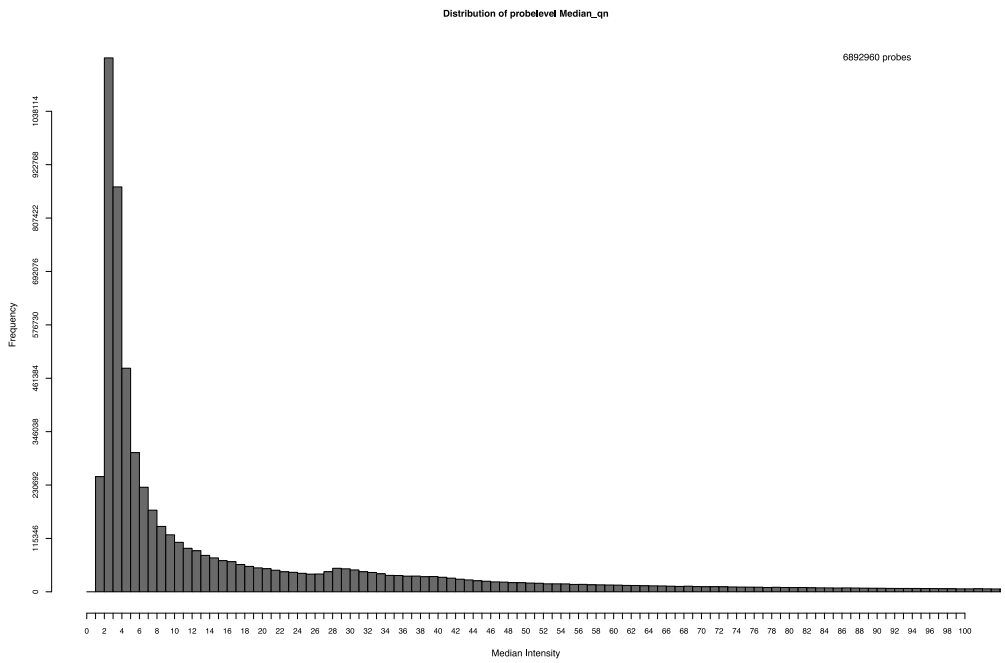


Figure 14. *Demonstrating the large peak of background probe signal seen in the 'raw' HTA 2.0 chip data in MP muscle samples.*

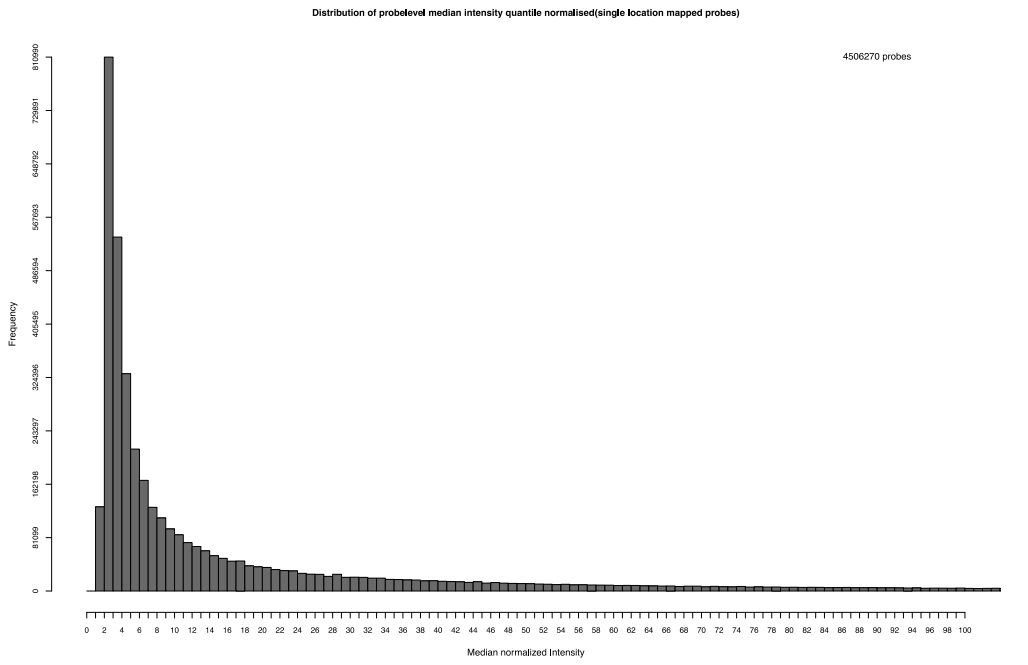


Figure 15*. Demonstrating the impact of re-alignment of the original HTA 2.0 probes to the latest genome sequence - ~2.4Million probes are removed.*

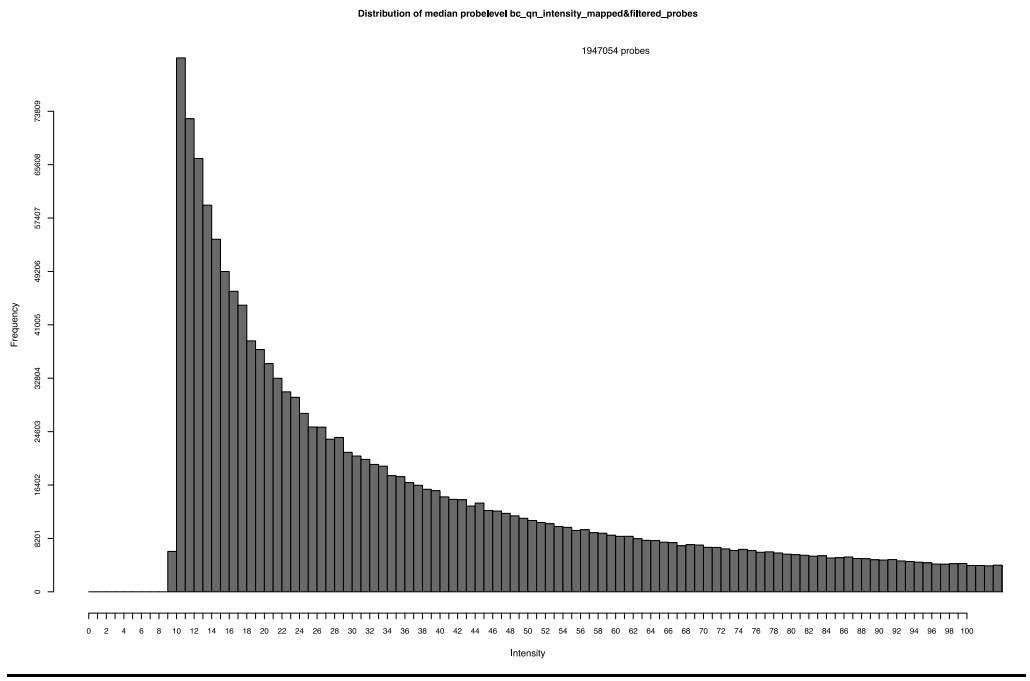


Figure 16. *Demonstrating the impact of processing the filtered HTA 2.0 probes to reflect the probes expressed in a tissue specific manner - ~2.3Million probes are removed.*

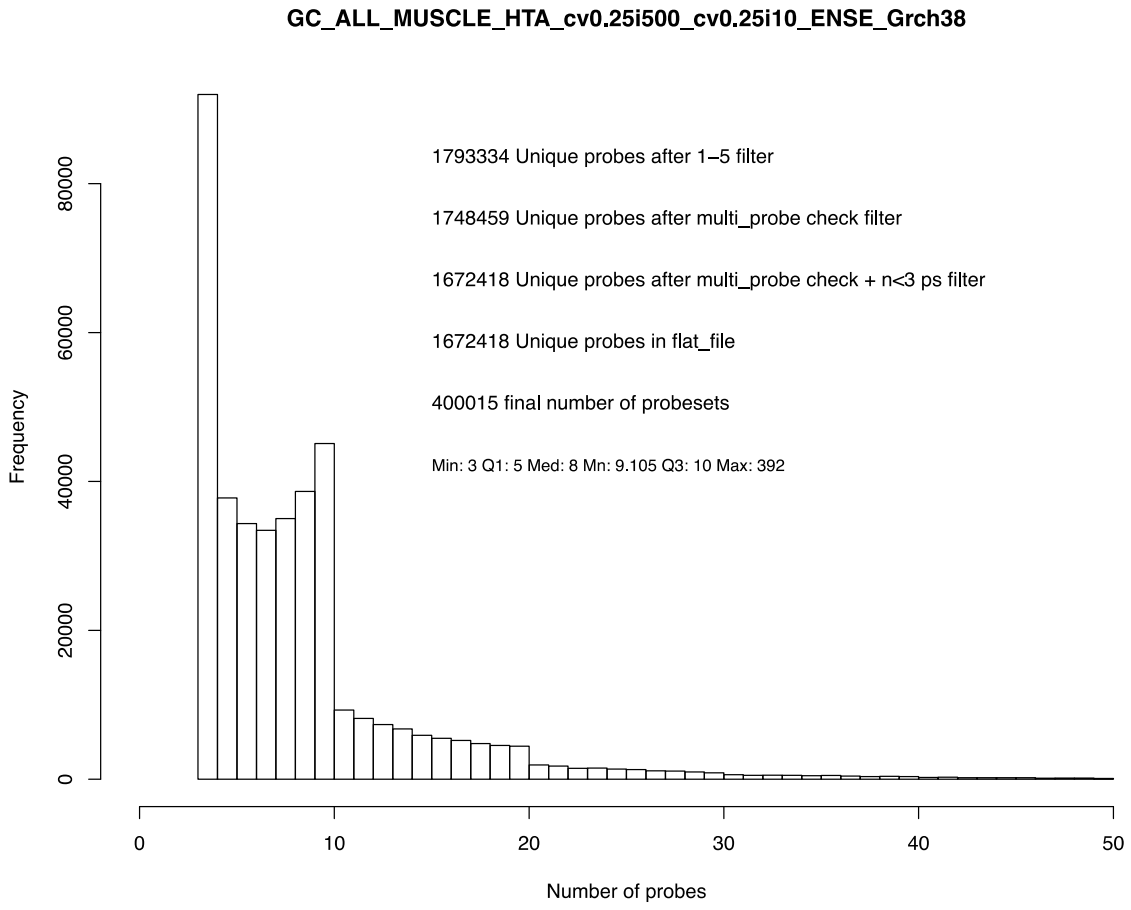


Figure 17. *A muscle specific HTA 2.0 'probe' map that is then assembled into probe-sets to detect individual exons. Only exons (probesets) with n=3 probes or more, aligned to 1 place in genome and containing probes above background signal are included.*

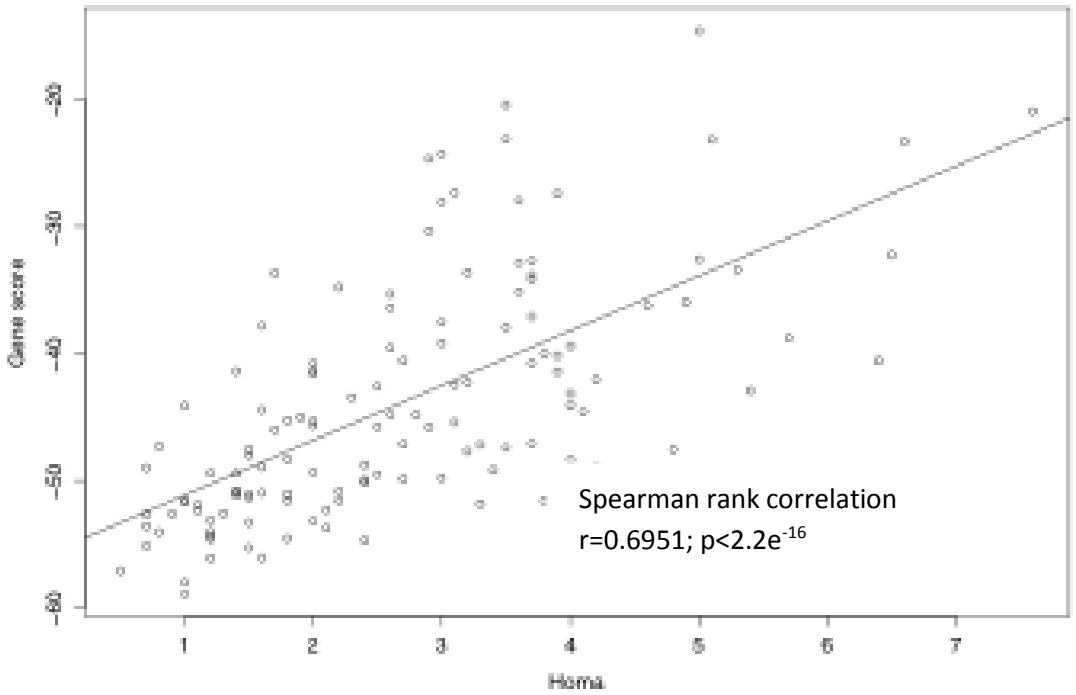


Figure 18. An example of a proto-type blood RNA versus HOMA linear model (Using STRRIDE II blood data). *This is a simple Knn + Ranking model was generated prior to custom CDF methodology and will be further developed to replicate across cohorts using the new linear modeling code and CDFS.*

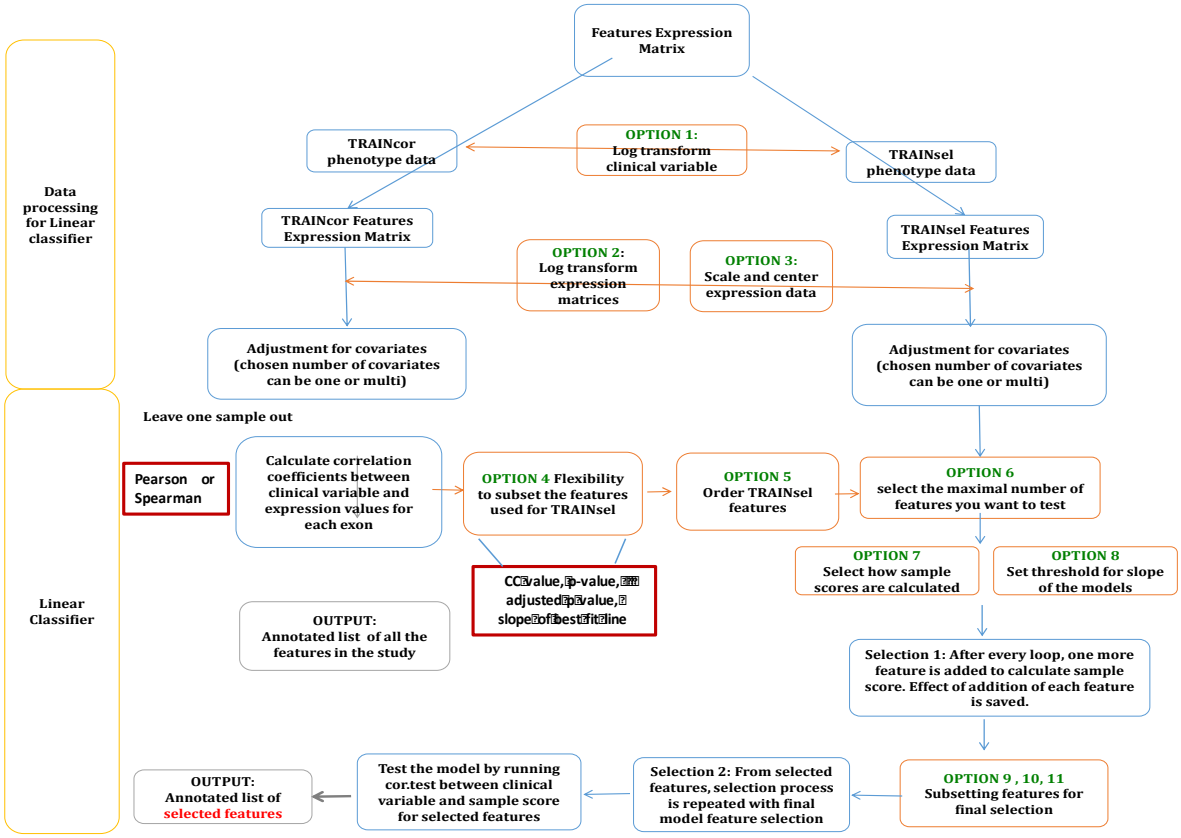


Figure 19. XRGenomics computational strategy for linear code

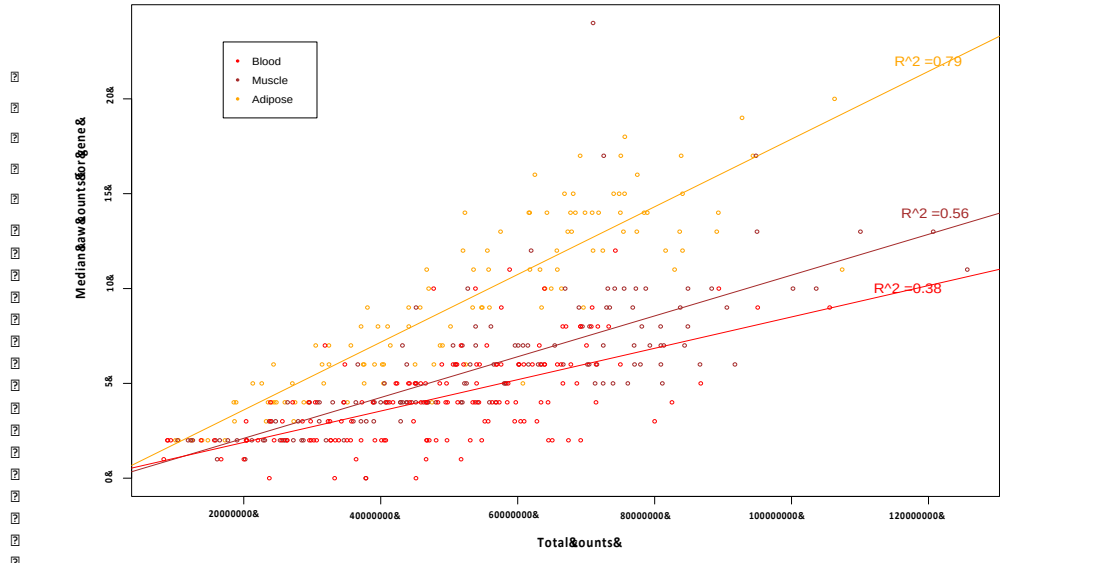


Figure 20. RNA sequencing not able to produce a strongly linear signal for lower expressed human tissue genes (especially muscle and blood).

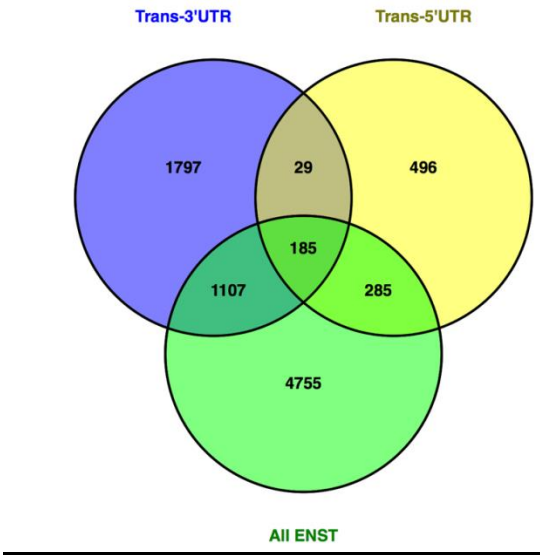


Figure 21. Full-Transcript, 3'UTR-transcript and 5'UTR-transcript regulation *in response to 6 weeks of 5by1 HIT (WP3) in the skeletal muscle of subjects that demonstrate a gain in aerobic capacity (FDR 10%, FC>1.2)*

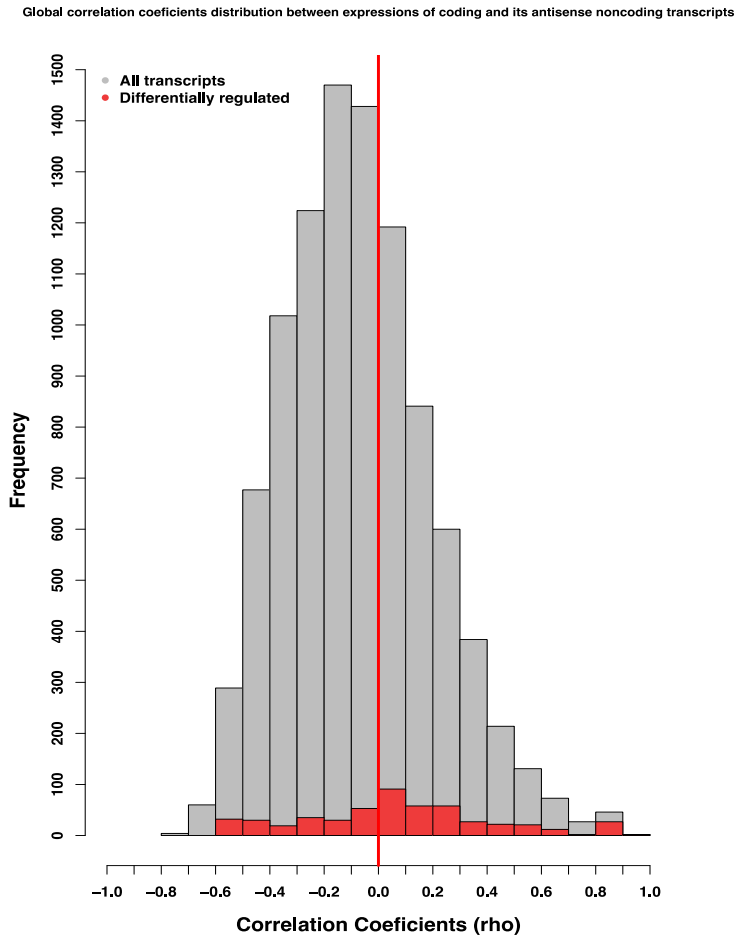


Figure 22. Global correlation *between lncRNA and CIS protein coding genes in human skeletal muscle (and HIT exercise regulated genes in red).*

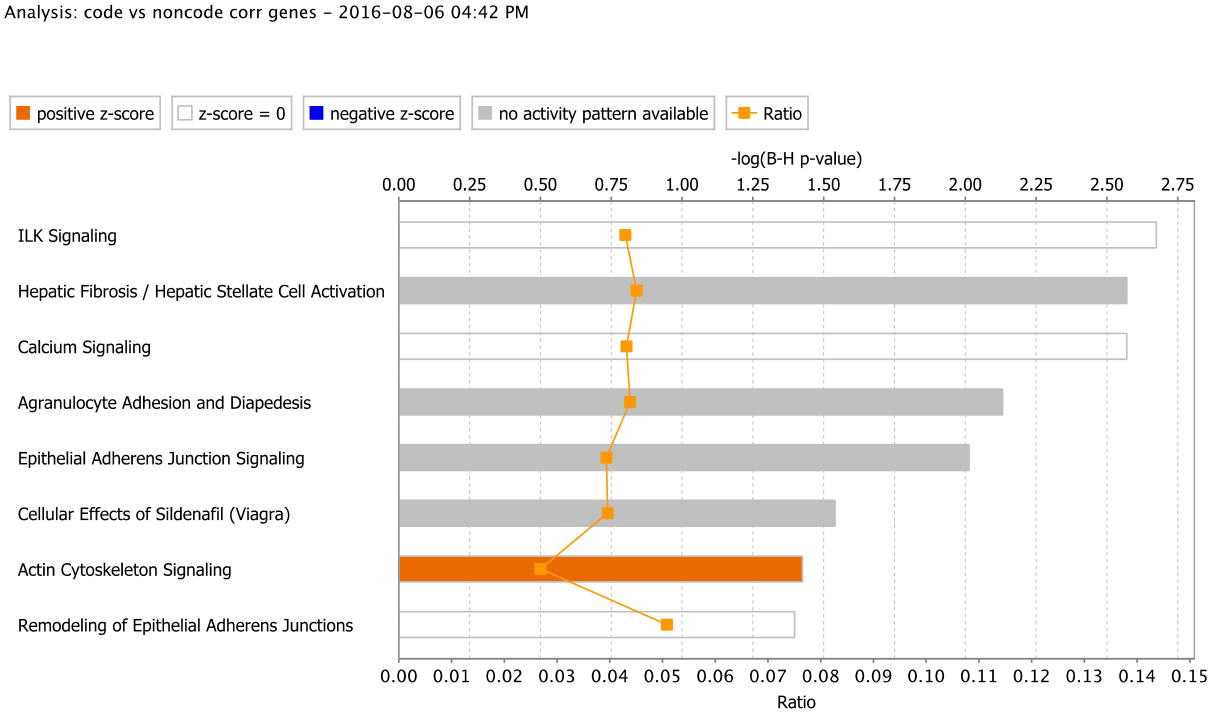


Figure 23. Up-stream regulators *associated with the protein-coding genes that are co-regulated with the lncRNA exercise responsive genes in subjects that demonstrate a robust improvement in aerobic capacity*

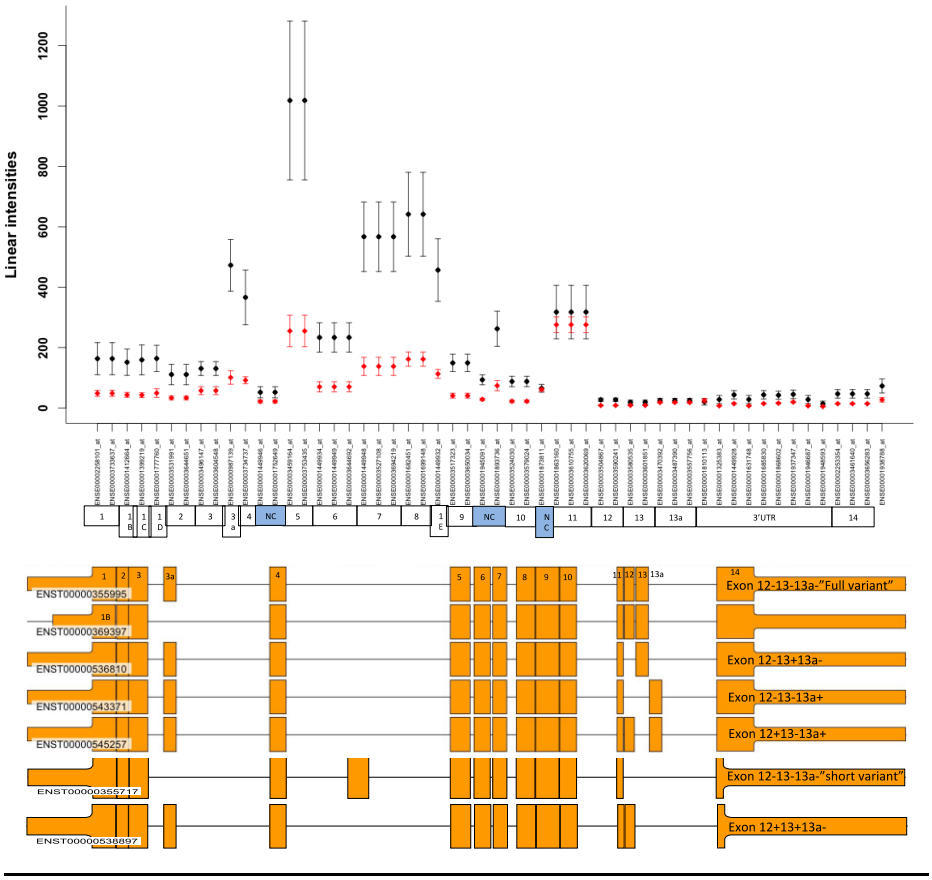


Figure 24. Probe-sets for TCF7L2 in muscle and adipose (WP5).

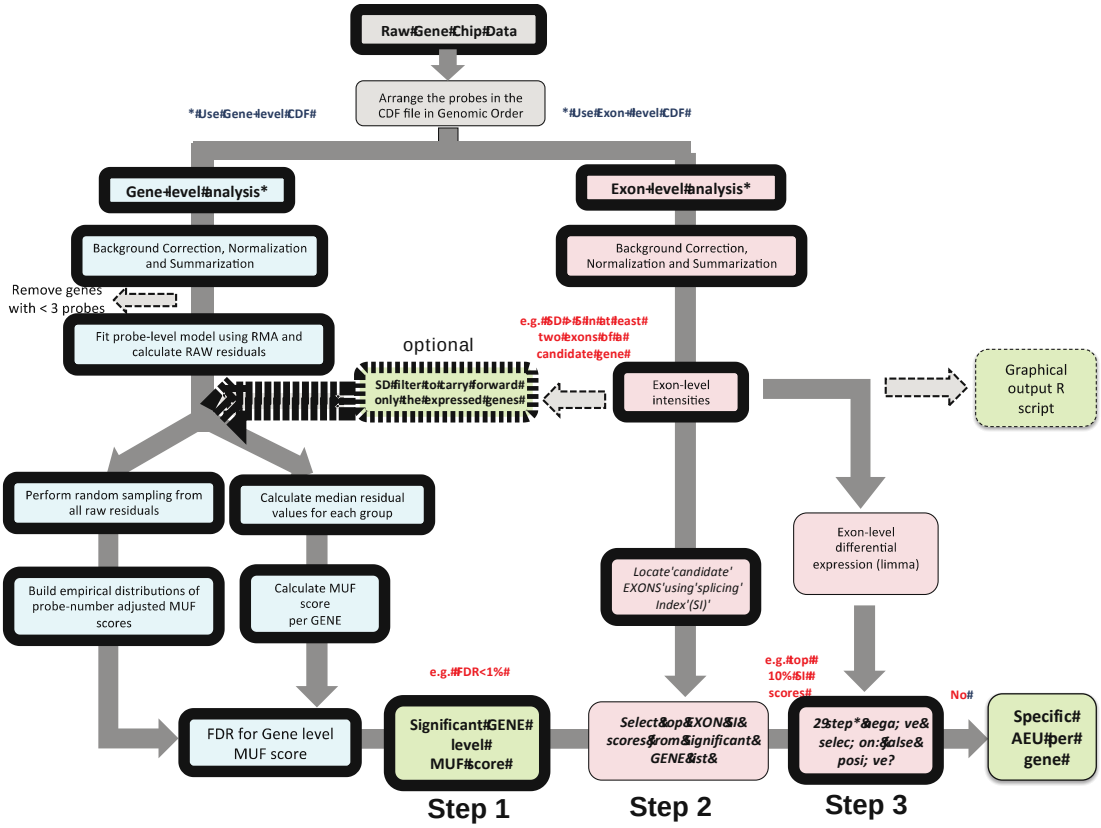


Figure 25. iGEMS analysis pipeline

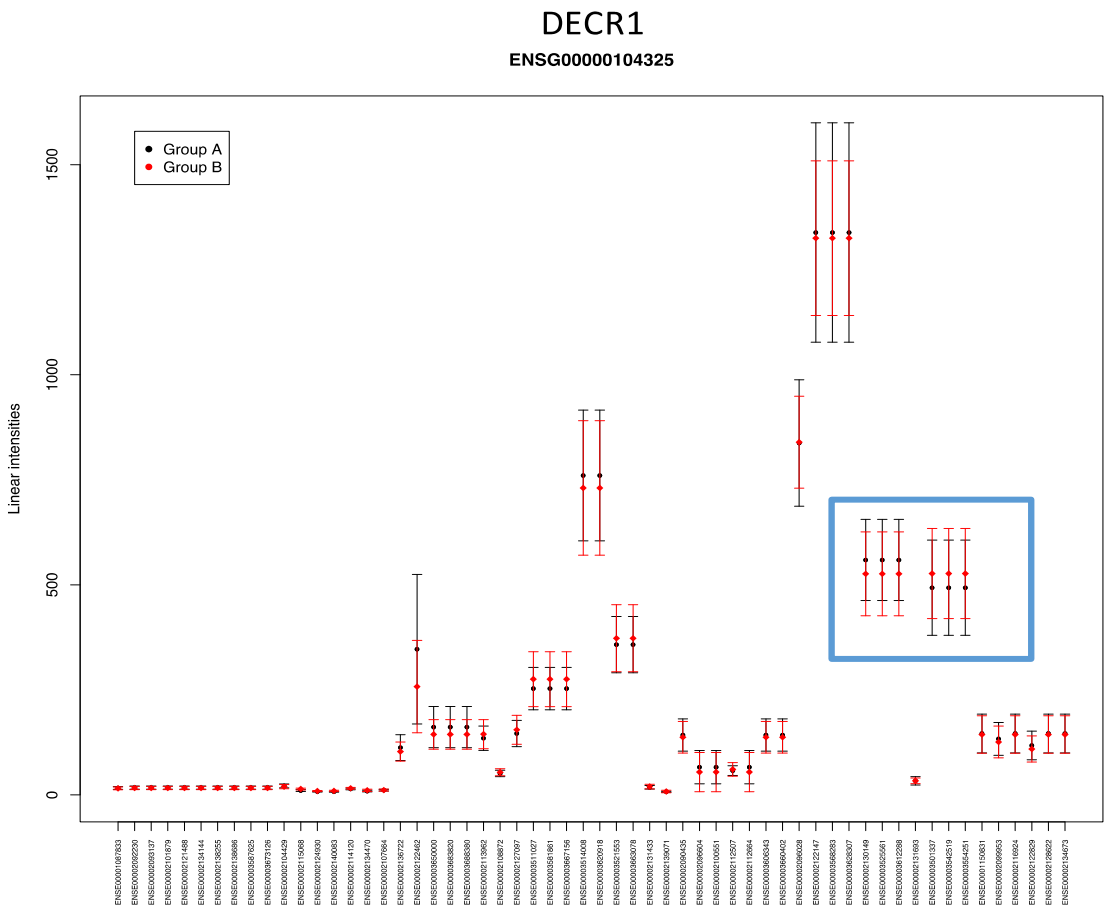


Figure 26. Plot of DECR1 in people with high versus low insulin sensitivity*. The blue box indicates alternative splicing for several Exons.*

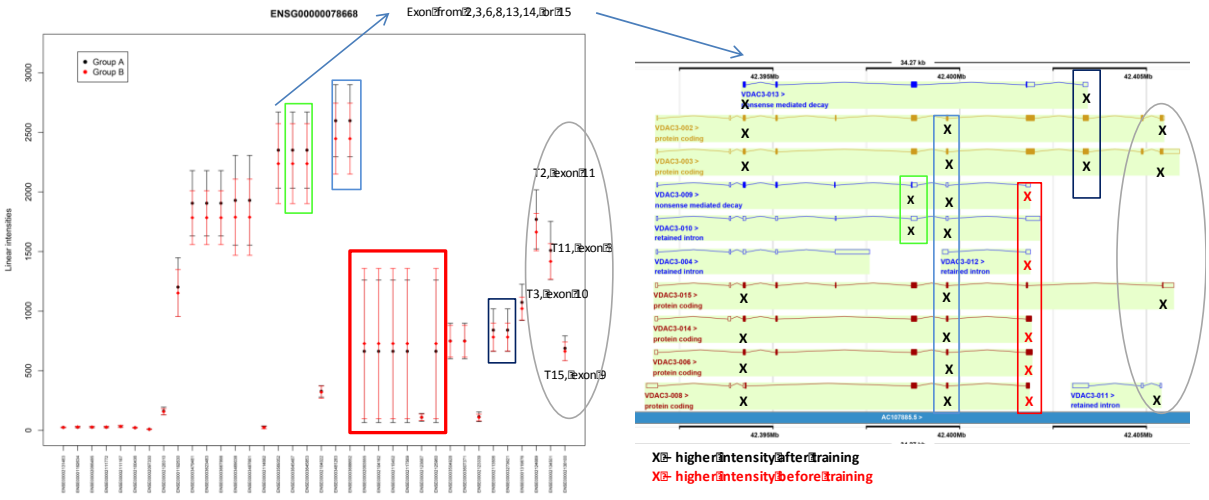


Figure 27. Application of iGEMS to muscle tissue related to high responders in VO2max

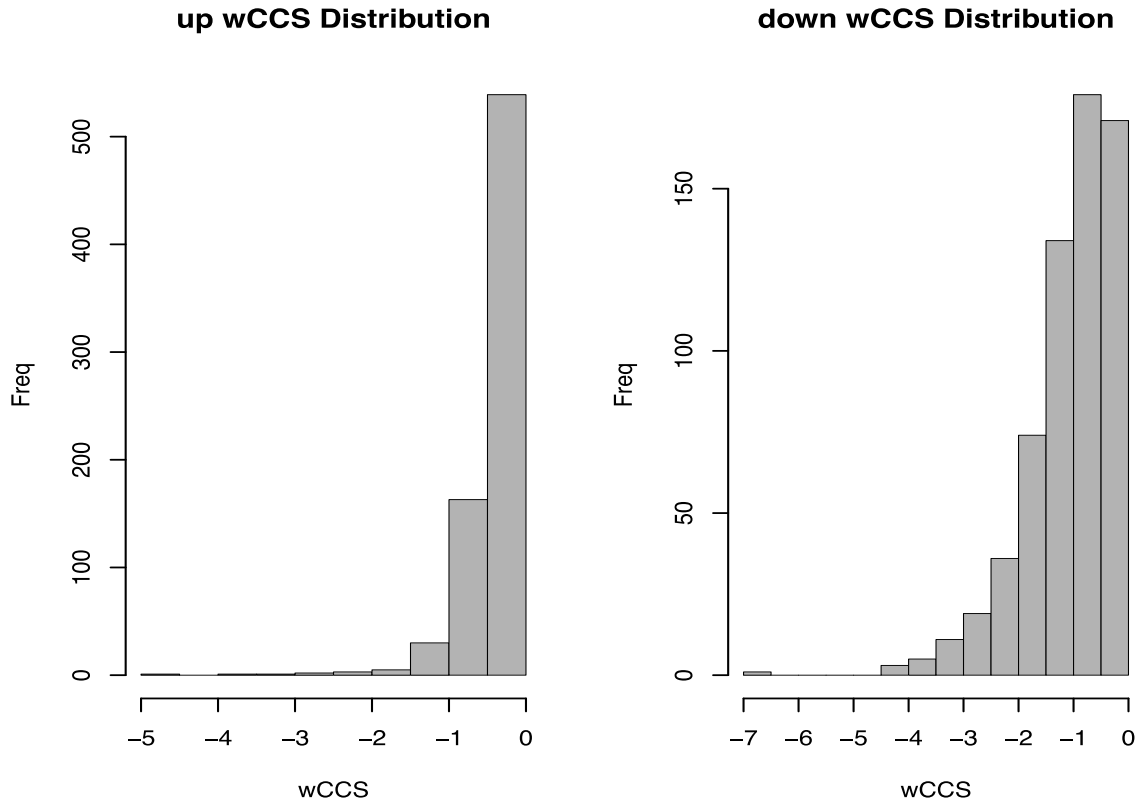


Figure 28. The wCCS for up and down regulated miRNAs WP5 D5.3 experiment.