# SUPPLEMENTARY METHODS

## Human physiological measurements

Before the study, the experimental protocol was explained to all subjects and informed written consent was obtained. Twenty-three young, sedentary volunteers were included, twelve males and eleven females. All subjects were healthy based on questionnaires and written consent, and non-smokers. Baseline physiological characteristics, including VO2-peak, are presented in Table 1.

Two one-legged knee-extension performance tests were conducted on a modified bicycle before and after the training period. A one-legged max test was performed using two different protocols; two minutes at a constant load (10W or 20W based on measured VO2-peak) followed by an increase in load by 3 or 5 watts every 30 seconds until exhaustion. A fifteen minutes one-legged optimal performance test (15-min test) was performed on a different day or at least 6h after the previous test. After one minute the load was 50% of the achieved maximum at the previous max test. Thereafter the load was kept as high as possible for the subject to be able to keep the pace at 60rpm for the full fifteen minutes. Heart rate was registered every minute, as well as a Borg-scale estimation of the effort of the working leg. Skeletal muscle biopsies from the *vastus lateralis* muscle were taken before and 24h after the last training session from both legs using a Bergstrom needle under local anesthesia,1 immediately frozen in liquid nitrogen and stored at -80°C until further analysis. The post-training performance tests were conducted 3-6 days after the biopsies.

The training regimen consisted of fully supervised one-legged knee-extension exercise training 4 times per week, 45 minutes each session. All subjects performed in total 45 exercise sessions, training only one randomized leg during the entire period (trained leg). The other leg was used as an untrained intraindividual control leg. There was a controlled increase in training load over the training period (Figure S1) and all subjects always trained at the same percentage of their 15-min maximal test. Repeated measures ANOVA was used to compare the effects of exercise training before and after the training period, as well as the interaction between the trained and untrained leg. Results are considered significant at p<0.05.

## Enzyme activity assays

The experimental procedure was done according to the fluorometric principles of Lowry & Passonneau.2 In brief, a section of a biopsy was freeze-dried and homogenized in 0.1 M phosphate buffer (pH 7.7) with 0.5% BSA. For citrate synthase (CS), the tissue lysates were added to a reagent solution (0.1 M Tris-HCl, 2.5 mM EDTA, 0.5 mM L-malate, 512.5 nM NAD+, 399μg MDH). 50 μg acetyl-CoA started the reaction and the velocity was registered with a fluorometer (reduction of NAD+ to NADH). A standard curve computed from known amounts of NADH was subsequently used to determine the CS. β-HAD activity was measured in a reagent solution (0,5M Imidazol (pH 7), 0.1M EDTA, 5mM NADH diluted in carbonate buffer, 2mM AcAcetyl-CoA) where addition of the tissue lysate started the reaction. Fluorecence of β-HAD oxidation of NADH to NAD+ was recorded and related to a standard curve.

## DNA methylation protocols

DNA was isolated with the Gentra Puregene Tissue Kit (Qiagen). In brief, 5-10 mg skeletal muscle tissue was homogenized in lysis buffer with proteinase K, and incubated at 55°Cfor 1h. After RNase A treatment, protein was precipitated followed by centrifugation at 14 000 x g. DNA was subsequently precipitated using ice-cold isopropanol. DNA was washed in 70% EtOH and mixed with 50ul of DNA hydration solution. After incubation at 65°C for 1h, the concentration and quality of the DNA was analyzed with Nanodrop® and gel electrophoresis. Genome-wide DNA methylation profiling was generated with the Infinium HumanMethylation450K BeadChip array (including 485,577 sites) on bisulfite-treated DNA,3 from sample before training (T1) and after training from the trained leg (T2) for 17 subjects in total. All samples were run at the BEA core facility, Karolinska Institutet. Bisulfite pyrosequencing was adopted for technical validation of the array data and the untrained leg was also included in the analysis. 7 sites in total were selected (3 increasing in methylation, 3 decreasing and 1 non-changing site, NCS). Primers were designed using the PyroMark assay design software (Qiagen) or the BiSearch primer design tool (<http://bisearch.enzim.hu>). Primer sequences are given below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **CpG site** | **Gene** | **Forward primer** | **Reverse primer** | **Sequencing primer** |
| cg13084335 | CRYAB | TGTGATTAGGGATTTGGTAATGTGATA | ACCTACTTAAAATTCCTAACTCTATACC | GAGTTAAGAAAAGAGAGATATAAA |
| cg19589060 | HK3 | AGTATTGTAGAGGGAATGGTTTAA | CCACTTACCAAACATCAACTAATT | GAATGGTTTAAAATTTTAGATTTAG |
| cg18928737 | IGFBP4 | GTAGTTTGGAAGTTGTAGTAGG | ACAACCCCTTAACTTCCC | AGGAGTTTAGAGTGTTTTTGAAT |
| cg22108292 | MYH3 | GGGGTTTTTATAGGTTATTGGTAATT | ACTTAAAAAAACCACTCATCCTCTTAC | GGTTTTTATAGGTTATTGGTAATTT |
| cg22740895 | MYOM2 | GTATTTTTAGGTGAAGGTGGAGTAGG | ATCTTCTCCCCCCCATACTTATTCTT | AGTAAGTTAGTATGATTATTAAAGG |
| cg22550299 | THBS2 | AGAAGTAGTGGTAGTAGTTAAT | TAACCTAAAAAAAACAACCC | TCCACCCTAACCTCTAA |
| cg12496014 | IGFBP4 (NCS) | TTTTAAGAATGTAAATGGGGTAG | ACCACACCCAACTAATTTTTATA | GGGTAGGGTATA GTGGTT |

## RNA sequencing

Total RNA was isolated based on the Trizol® method (Invitrogen, Carlsbad, CA, USA). The concentration and quality was determined with RNA 6000 Nano chip on the 2100 Bioanalyzer automated electrophoresis system (Agilent Technologies Inc., Santa Clara, CA, USA). Two μg of total RNA was used for the library preparation for each sample. The libraries were subsequently clustered on a cBot cluster-generation system according to the manufacturer’s instructions. The RNA libraries were sequenced as paired-end, 2x100bp on an Illumina HIseq2000 and generated an average of 21 million paired-end reads per sample.

## Bioinformatics analysis of DNA methylation data

We started using raw signals from the array measurements. We employed a pre-processing and normalization pipeline as reported elsewhere.4 DNA methylation levels β and M are defined as follows:

(1) 

(2) 

where IM and IU represent the fluorescence intensity originating form methylated or unmethylated CpG locus, respectively, and α is a constant. Briefly, lumi and “methylumi” packages were used for quality control and normalization. We filtered-out: the assays measuring 65 SNPs, probes on chromosomes X and Y and probes with a detection p value > 0.01 exceeding 5% of the samples. Color-bias adjustment and quantile normalization were performed on signal intensities. We then performed probe type bias adjustment using BMIQ normalization on β-values.5 Finally, we corrected for batch effect using the ComBat function. Differentially methylated positions (DMPs) were defined using limma package on M values, including the group (T2 vs. T1) and the subject as covariates. Given the paired design, the gender cannot be included as an additional covariate. For the contrast T2 vs. T1 (i.e. the training intervention), we estimated the effect size (difference in M-values or ΔM), p-values and FDR. DMP were selected if FDR<0.05. For sample clustering, principal component analysis was performed using only data from the DMPs.

## Annotation of DMPs

Standard Illumina annotation was used to annotate methylation data. For each annotation category (relation to genes, relation to CpG islands, enhancers) the relative fraction of positions located within each feature type was calculated for DMPs, non-DMPs and the entire array. NIH roadmap epigenomic data for skeletal muscle (GEO accession: GSM621637, GSM621640, GSM621641, GSM621679, GSM621680, GSM621685, GSM621686, GSM621694, GSM669948, GSM916064) were used to call H3K4me1, H3K4me3 and H3K27ac peaks using MACS. Replicate samples were merged to obtain a consensus peak list. A list of putative promoters (H3K4me3) enhancers (H3K4me1) and active enhancers (H3K27ac ∩ H3K4me1) was obtained and the relative fractions located within those elements were calculated as above. Furthermore, ChromHMM chromatin segmentation tracks for HSMM cell line were downloaded from UCSC (wgEncodeBroadHmmHsmmHMM) and the relative fraction falling in each segment category was calculated for with DMPs and the entire array position; a fold change was then obtained (DMPs vs. array).

## Functional enrichment of DMPs

GREAT (http://great.stanford.edu) was used to discover functional categories associated with DMPs. Using a “basal plus extension” DMP-gene association rule, we defined as enriched categories those showing a binomial FDR<0.05, a hypergeometric FDR<0.05 and a fold enrichment > 2. The enrichment for known transcriptional motifs was tested with HOMER (http://homer.salk.edu/homer/). Briefly, each DMP was extended bidirectionally 100 bp and the resulting intervals were used as input. Results were considered significant if p<10-10. Motifs were clustered using STAMP (http://www.benoslab.pitt.edu/stamp/index.php).

## Bioinformatics analysis of RNAseq data

After quality control (fastQC) and appropriate trimming (trim galore), mapping was performed using TopHat2 using GRCh37 genome and transcriptome models. After PCR duplicate removal (Picard), gene counts were obtained using HTSeq. EdgeR was used to extract differentially expressed genes (DEGs).6 Briefly, lowly-expressed genes were filtered-out (Counts Per Million (CPM) < 1 in at least half of the samples) and normalization factors were calculates using TMM strategy. Dispersion was estimated using a negative binomial model before testing for DEG using generalized linear models. We included the group (T2 vs. T1), the library preparation and the individual as covariates. Given the paired design, the gender cannot be included as an additional covariate. For the contrast T2 vs. T1 (i.e. the training intervention), we estimated the effect size (log2FC), p-values and FDR. DEG were selected if FDR<0.05. For sample clustering, a set of 1000 top genes was chosen to have largest biological variation between samples (largest tagwise dispersion). Then the distance between each pair of samples is the biological coefficient of variation as defined in edgeR. This distance was used to obtain a multidimensional scaling plot. Gene ontology analysis of RNA-seq data was done taking length bias into account, as implemented in goseq,7 and enriched KEGG patways were visualized with pathview.8

## Correlation analysis between DNA methylation and transcriptomics

A list of genes and the corresponding measured DNA methylation positions was obtained using standard Illumina annotation for 450k arrays. Spearman correlation was calculated for each pair, comparing the normalized CPM and the M-values respectively. The distribution of Spearman rho statistics between DNA methylation and gene expression was calculated either including all pairs of genes/methylation positions or only pairs formed by a DMP and a DEG. A Q-Q plot was obtained by plotting the observed p-values from the above correlation analysis against those obtained under a uniform distribution.

## Transcriptional network reconstruction

Transcriptional networks were reconstructed from gene expression data applying the Mutual Information (MI) method developed in ARACNE.9 MI between differentially expressed genes was considered significant if P<10-14. DPI criterion was applied to transcription factors only. Consensus networks were generated using bootstrap runs and a P<10-10. Small networks with less than five nodes were filtered out. The resulting network components were visualized and analyzed with Cytoscape applying the force-directed layout. For each independent network domain, gene ontology was tested using the BiNGO tool (<http://www.psb.ugent.be/cbd/papers/BiNGO/Home.html>) .

## References

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