

SUPPLEMENTARY MATERIAL

Table S1. Baseline participant characteristics. Height, weight, Body Mass Index, systolic BP, diastolic BP and heart rate are shown from the screening visit. Six-minute Walk test (6MWT) distance, Timed Up and Go test (TUG) time, grip strength and 36-Item Short Form Survey (SF-36) questionnaire are shown from visit 1. *One participant did not do the six-minute walk test because of fatigue after the other study procedures and is not included in the mean value.

Characteristic	Mean (range)	Characteristic	Mean (range)
Height (cm)	169.3 (154.4-183.5)	Quality of Life SF-36 (total)	99 (88-104)
Weight (kg)	70.0 (51.7-98.6)	SF-36 physical functioning	56 (5-100)
BMI	24.2 (18.9-30.9)	SF-36 role limitations due to physical health	28 (0-75)
Systolic BP (mmHg)	120 (96-148)	SF-36 role limitations due to emotional problems	54 (33-100)
Diastolic BP (mmHg)	70 (62-81)	SF-36 energy/fatigue	37 (20-75)
Heart rate (beats/min)	74 (16-89)	SF-36 Emotional well-being	61.5 (36-84)
6MWT (m)	320.5 (148.3-442.5)*	SF-36 Social functioning	56 (25-100)
Timed up and go (s)	13.6 (6.7-23.0)	SF-36 pain	53 (22.5-100)
Grip strength (kg)	23.8 (10.2-36.7)	SF-36 general health	34 (5-60)
Leg strength used for exercises in MRS (kg)	39.3 (23-59.5)		

Table S2. MitoSAlt analysis of skeletal muscle mtDNA before and after NR. Each participant had a different location of the single deletion. NR011 had the highest heteroplasmy in muscle and NR013 the lowest heteroplasmy. These results are shown graphically in Figure 3E and 3F. We note that the heteroplasmy levels calculated by MitoSAlt are higher than those measured by droplet digital PCR.

	Heteroplasmy in muscle at visit 1	Heteroplasmy in muscle at visit 5	Deletion site
NR011	78	65	8470-13446
NR012	65	44	7683-15017
NR013	39	15	6500-10968

Table S3. List of genes which show significant upregulation in muscle after four weeks of NR treatment. Genes which are included in MitoCarta 3.0 are indicated with *. MitoCarta 3.0 is an inventory of 1136 human genes encoding proteins with strong support for mitochondrial localisation.

Ensembl_gene_id	HGNC symbol	MitoCarta 3.0	Description (Source: HGNC)
ENSG00000099624	<i>ATP5F1D</i>	*	ATP synthase F1 subunit delta
ENSG00000250479	<i>CHCHD10</i>	*	coiled-coil-helix-coiled-coil-helix domain containing 10
ENSG00000178741	<i>COX5A</i>	*	cytochrome c oxidase subunit 5A
ENSG00000167996	<i>FTH1</i>	*	ferritin heavy chain 1
ENSG00000171766	<i>GATM</i>	*	glycine amidinotransferase
ENSG00000182512	<i>GLRX5</i>	*	glutaredoxin 5
ENSG00000125166	<i>GOT2</i>	*	glutamic-oxaloacetic transaminase 2
ENSG00000198804	<i>MT-CO1</i>	*	mitochondrially encoded cytochrome c oxidase I
ENSG00000198938	<i>MT-CO3</i>	*	mitochondrially encoded cytochrome c oxidase III
ENSG00000198840	<i>MT-ND3</i>	*	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 3
ENSG00000136521	<i>NDUFB5</i>	*	NADH:ubiquinone oxidoreductase subunit B5
ENSG00000165028	<i>NIPSNAP3B</i>	*	nipsnap homolog 3B
ENSG00000204370	<i>SDHD</i>	*	succinate dehydrogenase complex subunit D
ENSG00000075415	<i>SLC25A3</i>	*	solute carrier family 25 member 3
ENSG00000169021	<i>UQCRCF1</i>	*	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
ENSG00000078668	<i>VDAC3</i>	*	voltage dependent anion channel 3
ENSG00000185100	<i>ADSSI</i>		adenylosuccinate synthase 1

ENSG00000135334	<i>AKIRIN2</i>		akirin 2
ENSG00000198796	<i>ALPK2</i>		alpha kinase 2
ENSG00000171885	<i>AQP4</i>		aquaporin 4
ENSG00000146809	<i>ASB15</i>		ankyrin repeat and SOCS box containing 15
ENSG00000100628	<i>ASB2</i>		ankyrin repeat and SOCS box containing 2
ENSG00000151893	<i>CACUL1</i>		CDK2 associated cullin domain 1
ENSG00000113328	<i>CCNG1</i>		cyclin G1
ENSG00000118816	<i>CCNI</i>		cyclin I
ENSG00000104879	<i>CKM</i>		creatine kinase, M-type
ENSG00000175826	<i>CTDNEP1</i>		CTD nuclear envelope phosphatase 1
ENSG00000166266	<i>CUL5</i>		cullin 5
ENSG00000139914	<i>FITM1</i>		fat storage inducing transmembrane protein 1
ENSG00000130005	<i>GAMT</i>		guanidinoacetate N-methyltransferase
ENSG00000136732	<i>GYPC</i>		glycophorin C (Gerbich blood group)
ENSG00000167476	<i>JSRP1</i>		junctional sarcoplasmic reticulum protein 1
ENSG00000185028	<i>LRRC14B</i>		leucine rich repeat containing 14B
ENSG00000170860	<i>LSM3</i>		LSM3 homolog, U6 small nuclear RNA and mRNA degradation associated
ENSG00000108984	<i>MAP2K6</i>		mitogen-activated protein kinase kinase 6
ENSG00000169184	<i>MN1</i>		MN1 proto-oncogene, transcriptional regulator
ENSG00000168530	<i>MYL1</i>		myosin light chain 1
ENSG00000134986	<i>NREP</i>		neuronal regeneration related protein
ENSG00000149269	<i>PAK1</i>		p21 (RAC1) activated kinase 1
ENSG00000228801	<i>PCMTD1-DT</i>		PCMTD1 divergent transcript

ENSG00000171033	<i>PKIA</i>		cAMP-dependent protein kinase inhibitor alpha
ENSG00000006757	<i>PNPLA4</i>		patatin like phospholipase domain containing 4
ENSG00000123091	<i>RNF11</i>		ring finger protein 11
ENSG00000143171	<i>RXRG</i>		retinoid X receptor gamma
ENSG00000152700	<i>SAR1B</i>		secretion associated Ras related GTPase 1B
ENSG00000170624	<i>SGCD</i>		sarcoglycan delta
ENSG00000115593	<i>SMYD1</i>		SET and MYND domain containing 1
ENSG00000082146	<i>STRADB</i>		STE20 related adaptor beta
ENSG00000198252	<i>STYX</i>		serine/threonine/tyrosine interacting protein
ENSG00000113387	<i>SUB1</i>		SUB1 regulator of transcription
ENSG00000170417	<i>TMEM182</i>		transmembrane protein 182
ENSG00000178821	<i>TMEM52</i>		transmembrane protein 52
ENSG00000143549	<i>TPM3</i>		tropomyosin 3
ENSG00000170035	<i>UBE2E3</i>		ubiquitin conjugating enzyme E2 E3
ENSG00000198816	<i>ZNF358</i>		zinc finger protein 358

Table S4. List of genes which are significantly downregulated in muscle after four weeks of NR treatment. None of these genes are in MitoCarta 3.0.

Ensembl_gene_id	HGNC symbol	Description (Source: HGNC)
ENSG00000106772	<i>PRUNE2</i>	prune homolog 2 with BCH domain
ENSG00000107796	<i>ACTA2</i>	actin alpha 2, smooth muscle
ENSG00000189058	<i>APOD</i>	apolipoprotein D
ENSG00000111799	<i>COL12A1</i>	collagen type XII alpha 1 chain
ENSG00000099204	<i>ABLIM1</i>	actin binding LIM protein 1
ENSG00000131711	<i>MAP1B</i>	microtubule associated protein 1B
ENSG00000115306	<i>SPTBN1</i>	spectrin beta, non-erythrocytic 1

Table X Comparison of median values at baseline and after treatment in participants with single nucleotide variants (SNVs), participants with single large scale deletions (MtDel) and the whole cohort. P-values shown are from the Wilcoxon signed rank test (two-tailed). Interquartile ranges are shown in brackets.

Measure	SNVs baseline	SNVs Week 4	p	MtDel baseline	MtDel Week 4	p	Whole cohort baseline	Whole cohort week 4	p
COX deficient fibres									0.156
RRF									0.0935
CI/CS									0.547
CIV/CS									0.945
CS							1819 (609)	1828 (830)	0.64
Copy number	1312 (513)	2009 (845)	0.125	1972 (319)	1740 (743)	0.75	1611 (730)	1874 (900)	0.148
Heteroplasmy kPCr			0.625			0.50			NA
FGF-21 (pg/ml)							329 (107)	267 (115)	0.109
GDF-15 (pg/ml)							2016 (1235)	1994 (1221)	0.383
TUG (s)							11.9 (7.51)	9.96 (7.39)	0.04
6MWT (m)							360 (158)	352 (172)	0.58
Max grip strength (kg)							24.2 (12.3)	24.3, (18.6)	0.2
SF-36							101 (3)	94.5 (9.5)	0.16

Figure S1. Volcano plot showing up/downregulated genes after NR treatment. The red dots show genes which had significantly higher expression after 4 weeks of NR treatment (adjusted $p < 0.05$). The blue dots show genes which had significantly higher expression in the control sample taken before NR treatment. The labelled genes are also found in MitoCarta 3.0 and all of them had higher expression after 4 weeks of NR treatment.

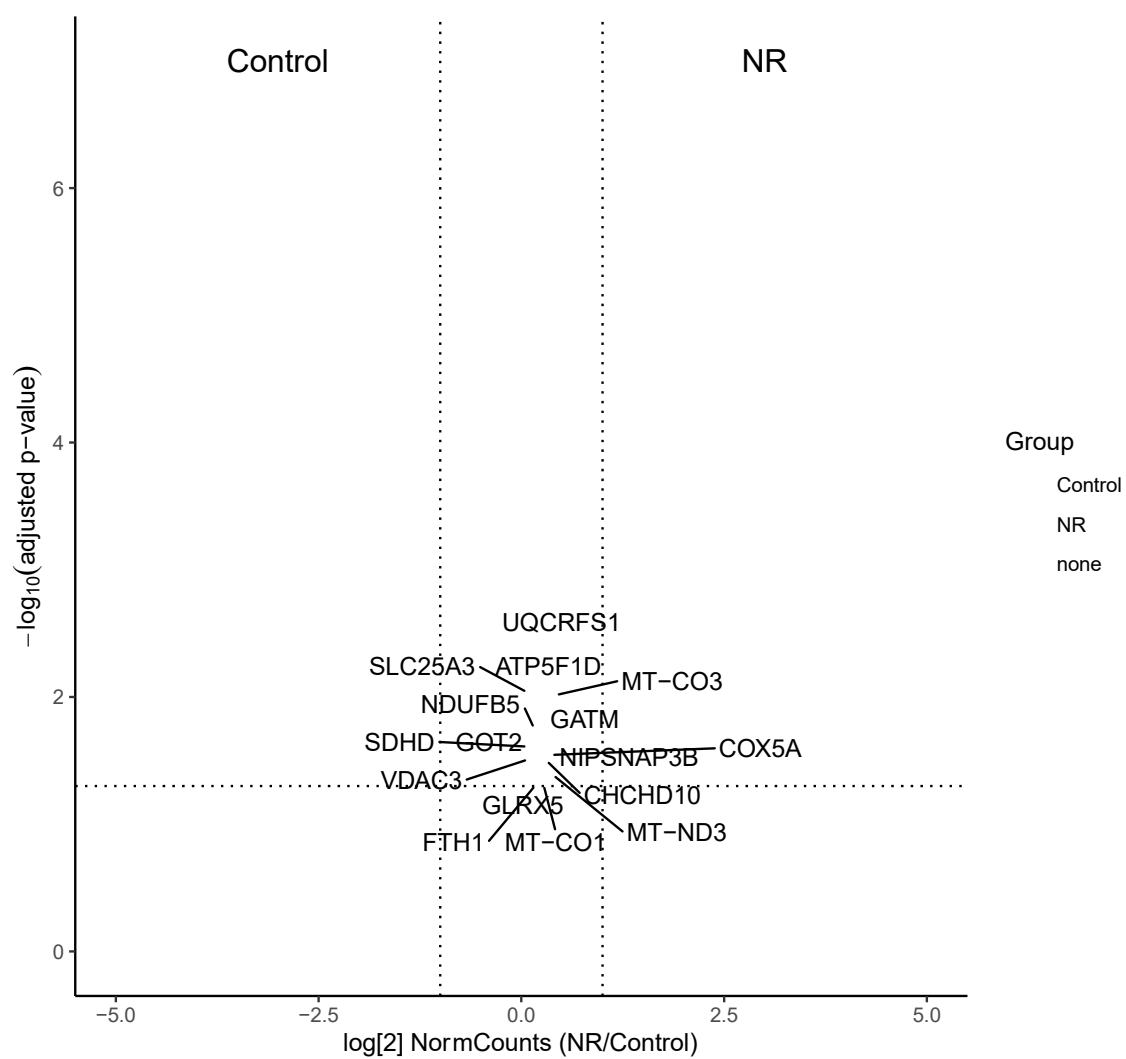


Figure S2. Pathway analysis of differentially expressed genes between visit 1 and visit 5 in muscle tissue

Gene Ontology (GO) pathway analysis was performed for the 62 differentially expressed genes in muscle. The most significantly enriched pathways are shown in dark blue. (A) GO Cellular Component. (B) GO Biological Process. (C) GO Molecular Function.

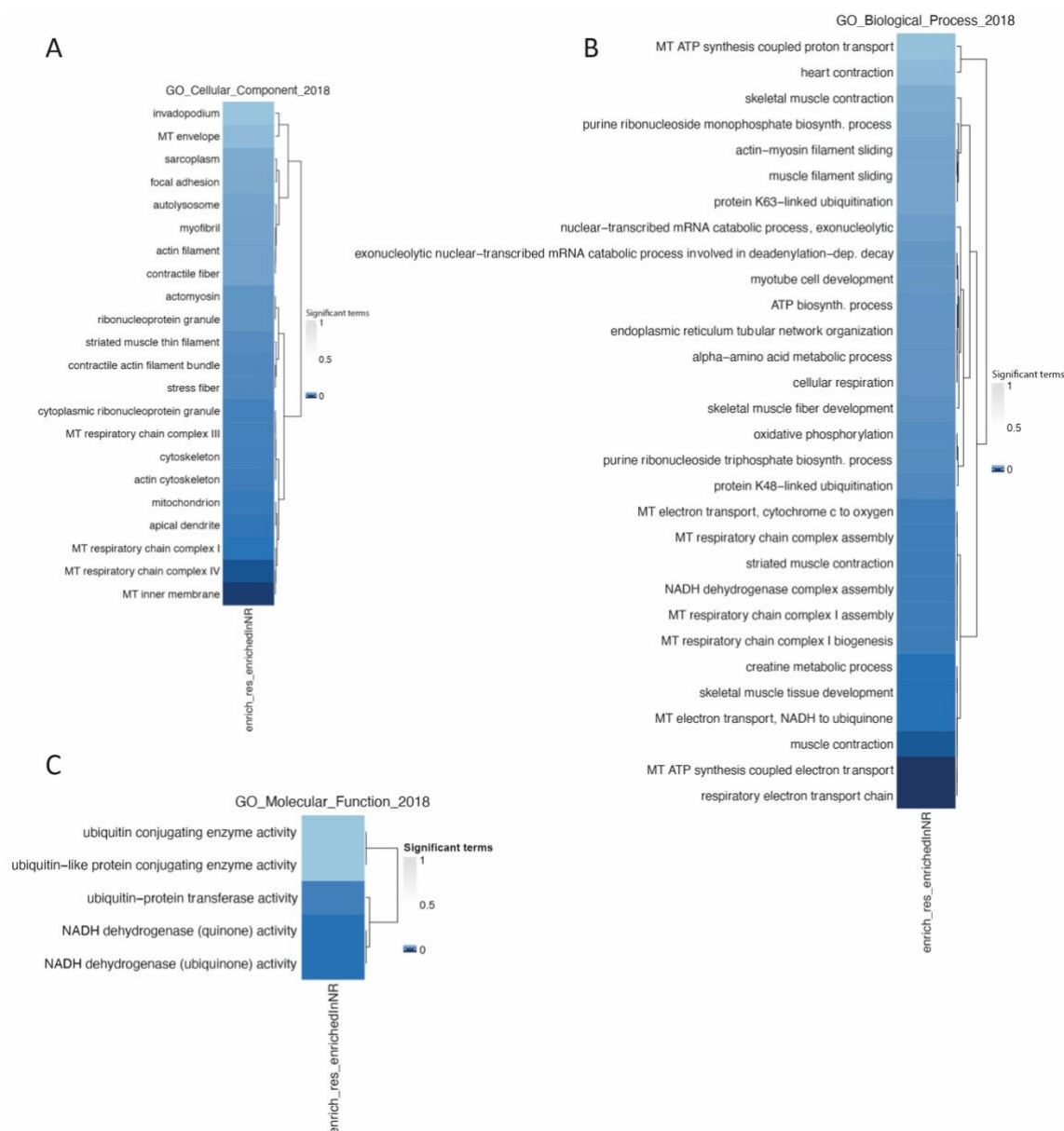


Figure S3. Volcano plot showing targeted metabolomics in muscle at visit 5 compared to visit 1. The plot shows the $-\log p$ value on the y axis and the log fold change on the x axis. The horizontal line corresponds to a p value of 0.05. Nicotinamide N oxide, 2,3-Diphosphoglyceric acid, 4PY (N¹-methyl-4-pyridone-3-carboxamide), and 2PY (N¹-methyl-2-pyridone-5-carboxamide) were increased after 4 weeks of NR treatment. The metabolites analysed were sugars and organic acids, polar metabolites in the folate cycle, methionine cycle and transsulfuration pathways and NAD related metabolites, phosphorylated metabolites and carnitines. A total of 220 metabolites were analysed.

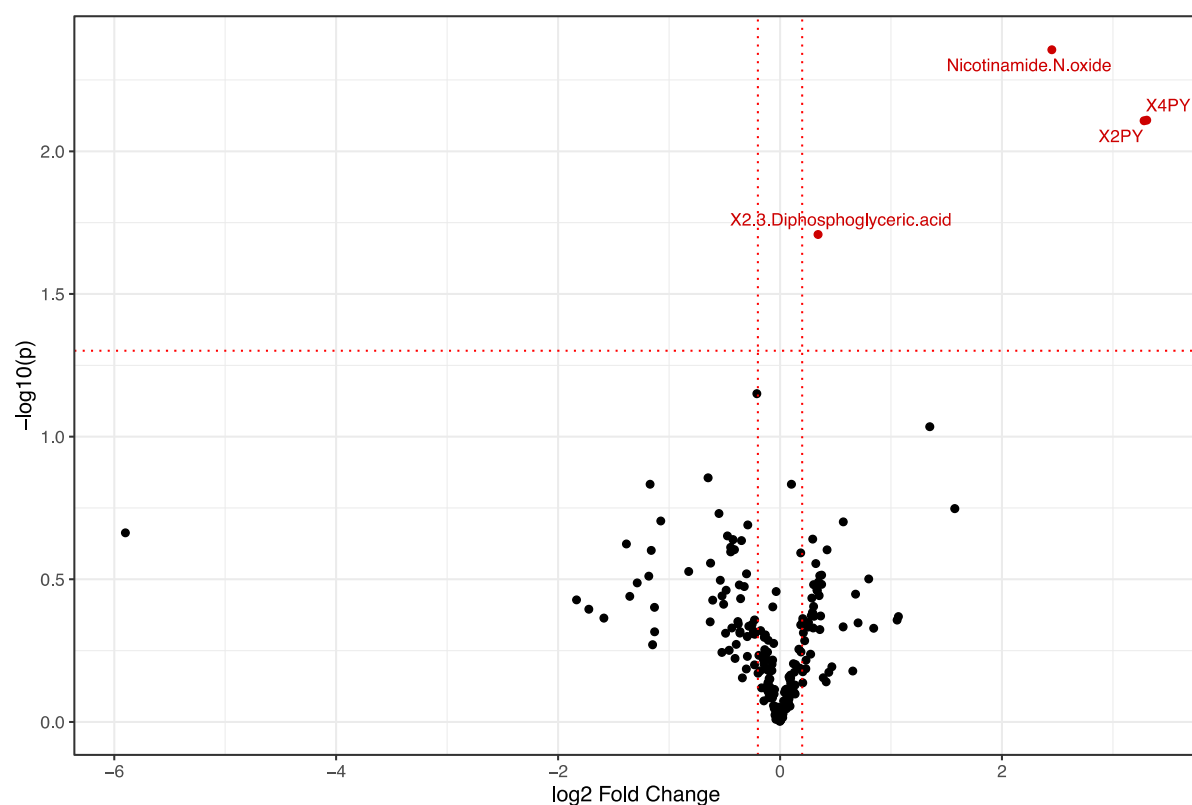


Figure S4. Integrated transcriptomic and proteomic analysis by genetic subgroup before and after NR. (A) Correlation plot showing the relationship between transcriptomic and proteomic datasets for SNV subgroup showing a correlation of **0.97** between omics layers. (B) Deletion subgroup showing a correlation of **0.99**. The plots were generated using the DIABLO framework, separately applied to each genotype group, with ellipses representing the distribution of samples before (V1) and after (V5) NR treatment. (C) Feature Loadings Plots for proteomics and transcriptomics in the SNV group, showing the top 10 proteins and transcripts contributing to the separation in the first component of the DIABLO analysis. (D) Feature Loadings Plots for proteomics and transcriptomics in the Deletion group, showing the top 10 proteins and transcripts contributing to the separation in the first component of the DIABLO analysis.

