

SUPPLEMENTARY INFORMATION

The resveratrol-enriched rice DJ526 boosts motor coordination and physical strength

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Supplementary Tables 1, 2, 3, 4

Supplementary Figure 1, 2, 3 and legends

Supplementary Tables

Supplementary Table S1: The compositional variation of the Formula Diet fed to the experimental mice

Ingredient	Ctrl	RS	DJ	DJ526
Casein	200	200	200	200
L-Cysteine	3	3	3	3
Corn Starch	397.486	397.486	0	0
Sucrose	100	100	0	0
Maltodextrin	132	132	132	132
Soybean Oil	70	70	70	70
Cellulose	50	50	50	50
Mineral Mix, AIN-93G-MX (94046)	35	35	35	35
Vitamin Mix, AIN-93-VX (94047)	10	10	10	10
Choline Bitartrate	2.5	2.5	2.5	2.5
TBHQ, Antioxidant	0.014	0.014	0.014	0.014
Resveratrol	0	0.00098	0	0
Dongjin Rice	0	0	497.486	0
Dongjin-526 Rice	0	0	0	497.486
Total (g)	1000	1000	1000	1000

All diet formulations were based on the diet formula of nonfat normal diet (TD.94045 AIN-93G) of Raonbio (Seoul, Korea), which is the control diet in this experiment. Deviations from the control are indicated in bold. The resveratrol concentration in all of the formula diets was quantified by HPLC (ACQUITY UPLC, Waters, MA, USA). The resveratrol quantities were 0.69 $\mu\text{g/g}$ in resveratrol-enriched DJ526 diet (DJ526) and resveratrol diet (RS) each. However, resveratrol was not detected in control diet (Ctrl) and dongjin rice diet (DJ).

Supplementary Table S2: Differentially expressed genes by effect of resveratrol, Dongjin rice, and the resveratrol-enriched rice DJ526 (DJ526)

Gene	Z ratios RS/Ctrl	Gene	Z ratios RS/Ctrl
Sult2a4	11.07	Pde5a	-11.02
Sult2a1	9.13	Dirc2	-6.80
Sult2a3	8.36	Serpina4-ps1	-6.73
Gm6168	7.30	Emilin3	-6.08
Sult2a6	6.77	Crygf	-5.55
Ap3b2	5.63	Dnahc7a	-5.53
2310038E17Rik	5.33	Gypa	-5.40
Gm11435	5.16	Bves	-4.71
Cd70	5.12	Olf681	-4.47
Olf1106	5.07	Pbx1	-3.93

Gene	Z ratios DJ/Ctrl	Gene	Z ratios DJ/Ctrl
Csf3	5.53	Duoxa1	-5.88
Sub1	5.36	Pnoc	-5.68
Olf467	5.03	Olf877	-4.49
Serp1b1c	4.95	EG668249	-4.48
Ccnb1ip1	4.93	Prdm14	-4.47
4930578C19Rik	4.93	Shh	-4.22
D330041H03Rik	4.35	Olf1513	-4.03
Dctd	4.28	2410012M07Rik	-4.00
H2-M5	4.21	Myh13	-3.71
Olf915	3.95	Chn1	-3.61

Gene	Z ratios DJ526/Ctrl	Gene	Z ratios DJ526/Ctrl
Sult2a6	5.65	Gpr31c	-7.46
Ap3b2	5.13	Spock1	-6.72
Tnfaip6	5.08	Olf638	-6.29
Tas1r2	4.88	Olf453	-6.03
Arsi	4.77	Fgf15	-5.97
Olf119	4.62	Gm4050	-5.76
Trim43b	4.57	Dnahc1	-5.73
H2-M5	4.39	2810049E08Rik	-5.64
Olf569	4.37	Gm14003	-5.42
Usp54	4.21	Gm4794	-5.19

The most highly significant up-regulated and down-regulated genes in the livers of mice on resveratrol diet (RS), dongjin rice diet (DJ) and resveratrol-enriched DJ526 diet (DJ526) towards those on a control diet (Ctrl) groups are shown. Genes were filtered using a cutoff Z-ratio ± 1.5 and P-value of < 0.05 . Genes meeting these criteria were ranked by Z-ratio.

Supplementary Table S3: The most highly significant up-regulated and down-regulated pathways in the livers of mice on resveratrol diet (RS), Dongjin rice diet (DJ) and the resveratrol-enriched DJ526 diet (DJ526) towards those on the control diet (Ctrl) groups

RS/Ctrl	
Up	gene_sets.gmt#BIOCARTA_NO2IL12_PATHWAY gene_sets.gmt#KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION gene_sets.gmt#REACTOME_KINESINS gene_sets.gmt#KEGG_TASTE_TRANSDUCTION gene_sets.gmt#PID_TCR_CALCIIUM_PATHWAY gene_sets.gmt#PID_IL12_2PATHWAY gene_sets.gmt#PID_IL12_STAT4_PATHWAY gene_sets.gmt#BIOCARTA_INFLAM_PATHWAY gene_sets.gmt#PID_CD8_TCR_DOWNSTREAM_PATHWAY gene_sets.gmt#BIOCARTA_CTLA4_PATHWAY gene_sets.gmt#PID_AURORA_B_PATHWAY gene_sets.gmt#BIOCARTA_NKT_PATHWAY
Down	gene_sets.gmt#BIOCARTA_PROTEASOME_PATHWAY gene_sets.gmt#HALLMARK_MYC_TARGETS_V1 gene_sets.gmt#REACTOME_DESTABILIZATION_OF_MRNA_BY_AUF1_HNRNP_D0 gene_sets.gmt#KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS gene_sets.gmt#REACTOME_REGULATION_OF_APOPTOSIS gene_sets.gmt#HALLMARK_MTORC1_SIGNALING gene_sets.gmt#REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G gene_sets.gmt#REACTOME_AUTODEGRADATION_OF_THE_E3_UBIQUITIN_LIGASE_COPI gene_sets.gmt#REACTOME_RORA_ACTIVATES_CIRCADIAN_EXPRESSION gene_sets.gmt#REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSOMES gene_sets.gmt#PID_ECADHERIN_KERATINOCYTE_PATHWAY gene_sets.gmt#HALLMARK_CHOLESTEROL_HOMEOSTASIS
DJ/Ctrl	
Up	gene_sets.gmt#KEGG_PRIMARY_IMMUNODEFICIENCY gene_sets.gmt#REACTOME_DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX gene_sets.gmt#PID_CD8_TCR_DOWNSTREAM_PATHWAY gene_sets.gmt#BIOCARTA_NO2IL12_PATHWAY gene_sets.gmt#ST_TUMOR_NECROSIS_FACTOR_PATHWAY gene_sets.gmt#PID_IL12_2PATHWAY gene_sets.gmt#REACTOME_BASE_EXCISION_REPAIR gene_sets.gmt#REACTOME_NEF_MEDIATES_DOWN_MODULATION_OF_CELL_SURFACE_RECEPTORS_BY_RECRUITING_THEM_TO_CLATHRIN_ADAPTERS gene_sets.gmt#KEGG_BASE_EXCISION_REPAIR gene_sets.gmt#PID_AURORA_B_PATHWAY gene_sets.gmt#BIOCARTA_TNFR2_PATHWAY gene_sets.gmt#BIOCARTA_HIVNEF_PATHWAY
Down	gene_sets.gmt#KEGG_MATURITY_ONSET_DIABETES_OF_THE_YOUNG gene_sets.gmt#REACTOME_SIGNALING_BY_NODAL gene_sets.gmt#REACTOME_REGULATION_OF_GENE_EXPRESSION_IN_BETA_CELLS gene_sets.gmt#PID_CONE_PATHWAY gene_sets.gmt#KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES gene_sets.gmt#PID_RHODOPSIN_PATHWAY gene_sets.gmt#BIOCARTA_P53HYPOXIA_PATHWAY gene_sets.gmt#PID_PI3K_PLC_TRK_PATHWAY gene_sets.gmt#BIOCARTA_SHH_PATHWAY gene_sets.gmt#REACTOME_ACTIVATED_AMPK_STIMULATES_FATTY_ACID_OXIDATION_IN_MUSCLE gene_sets.gmt#REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT gene_sets.gmt#BIOCARTA_PGC1A_PATHWAY

DJ526/Ctrl

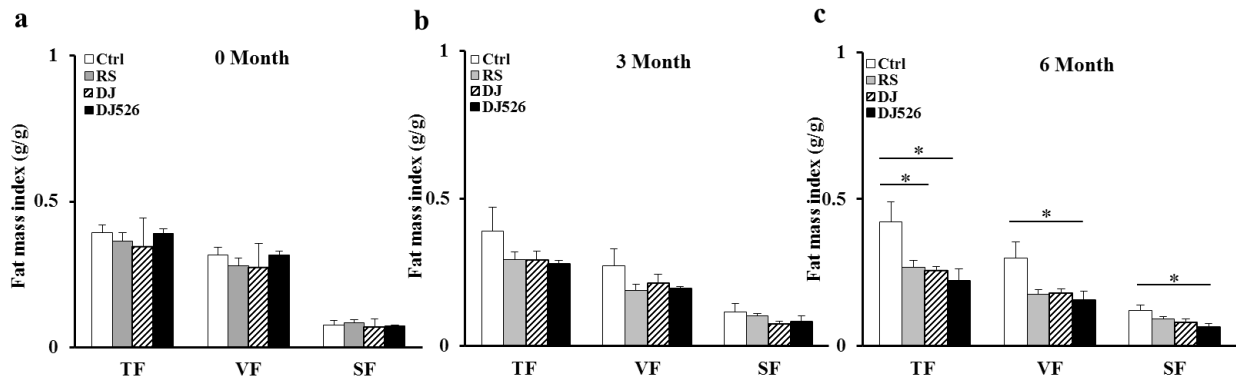
Up	gene_sets.gmt#KEGG_TASTE_TRANSDUCTION gene_sets.gmt#KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION gene_sets.gmt#PID_CD8_TCR_DOWNSTREAM_PATHWAY gene_sets.gmt#PID_NFAT_TFPATHWAY gene_sets.gmt#HALLMARK_E2F_TARGETS gene_sets.gmt#REACTOME_ERK_MAPK_TARGETS gene_sets.gmt#KEGG_GRAFT_VERSUS_HOST_DISEASE gene_sets.gmt#REACTOME_NUCLEAR_EVENTS_KINASE_AND_TRANSCRIPTION_FACTOR_ACTIVATION gene_sets.gmt#PID_AURORA_B_PATHWAY gene_sets.gmt#REACTOME_SYNTHESIS_SECRETION_AND_INACTIVATION_OF_GLP1 gene_sets.gmt#REACTOME_INCRETIN_SYNTHESIS_SECRETION_AND_INACTIVATION gene_sets.gmt#PID_PLK1_PATHWAY
Down	gene_sets.gmt#KEGG_OXIDATIVE_PHOSPHORYLATION gene_sets.gmt#HALLMARK_FATTY_ACID_METABOLISM gene_sets.gmt#KEGG_PPAR_SIGNALING_PATHWAY gene_sets.gmt#KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS gene_sets.gmt#REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS gene_sets.gmt#REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS gene_sets.gmt#REACTOME_RESPIRATORY_ELECTRON_TRANSPORT gene_sets.gmt#REACTOME_TRIGLYCERIDE_BIOSYNTHESIS gene_sets.gmt#PID_SMAD2_3PATHWAY gene_sets.gmt#HALLMARK_ANGIOGENESIS gene_sets.gmt#HALLMARK_OXIDATIVE_PHOSPHORYLATION gene_sets.gmt#REACTOME_LATENT_INFECTION_OF_HOMO_SAPIENS_WITH_MYCOBACTERIUM_TUBERCULOSIS

Supplementary Table S4: List of primer used for quantitative real-time PCR

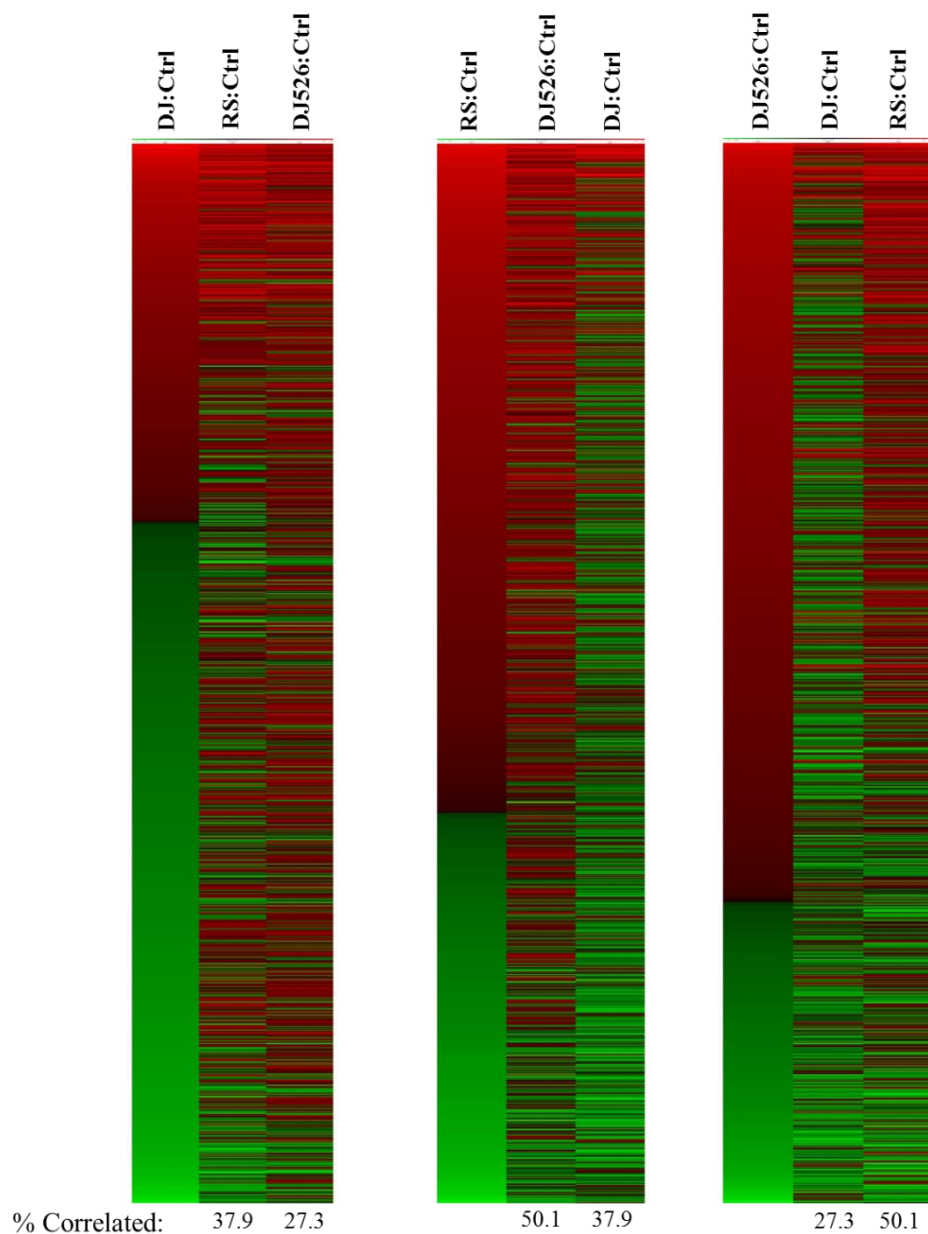
Primer name	Accession No.	Nucleotide Sequence (5'→3')	5' Position	Target length
Sult2a4-F	NM_001101534.1	TCCAGGGTCACTCAGAACTT	423	72
Sult2a4-R	NM_001101534.1	TGCTCAAACCATGATCCATA	494	72
Ap3b2-F	NM_021492.3	TTCAAGAAGGAACAGGGAAA	3249	70
Ap3b2-R	NM_021492.3	AGGTGTCTGGCAATGTAAGC	3318	70
Duoxa1-F	NM_145395.2	CTGGTAGCCACTGAGTTTCC	1312	70
Duoxa1-R	NM_145395.2	GGACAGTTCTGCTGGTTTTG	1381	70
Pnoc-F	NM_010932.2	GCCCCATCTTCTCACTCATC	1170	70
Pnoc-R	NM_010932.2	CCCAGGTCTGATTTCATGTT	1239	70
Sub1-F	ENSMUST00000110504	CGGACAGCGAAGTTGAAAAA	164	70
Sub1-R	ENSMUST00000110504	TGCTTCTTCACGGGCTTCTC	233	70
Sult2a1-F	NM_001111296.2	CCAGGGTCACTCGGAACTTA	481	70
Sult2a1-R	NM_001111296.2	GCTCAAACCATGATCCGAAT	550	70
Sult2a6-F	NM_001081325.2	GAACGTTACCCCTGGCTAGA	254	70
Sult2a6-R	NM_001081325.2	TGAGTCGTGGTCCTTCCTTA	323	70
GAPDH-F	NM_008084.3	GGCATTGCTCTCAATGACAA	1132	95
GAPDH-R	NM_008084.3	ATGRAGGCCATGAGGTCCAC	1226	95

F, forward; R, reverse

Supplementary Figures

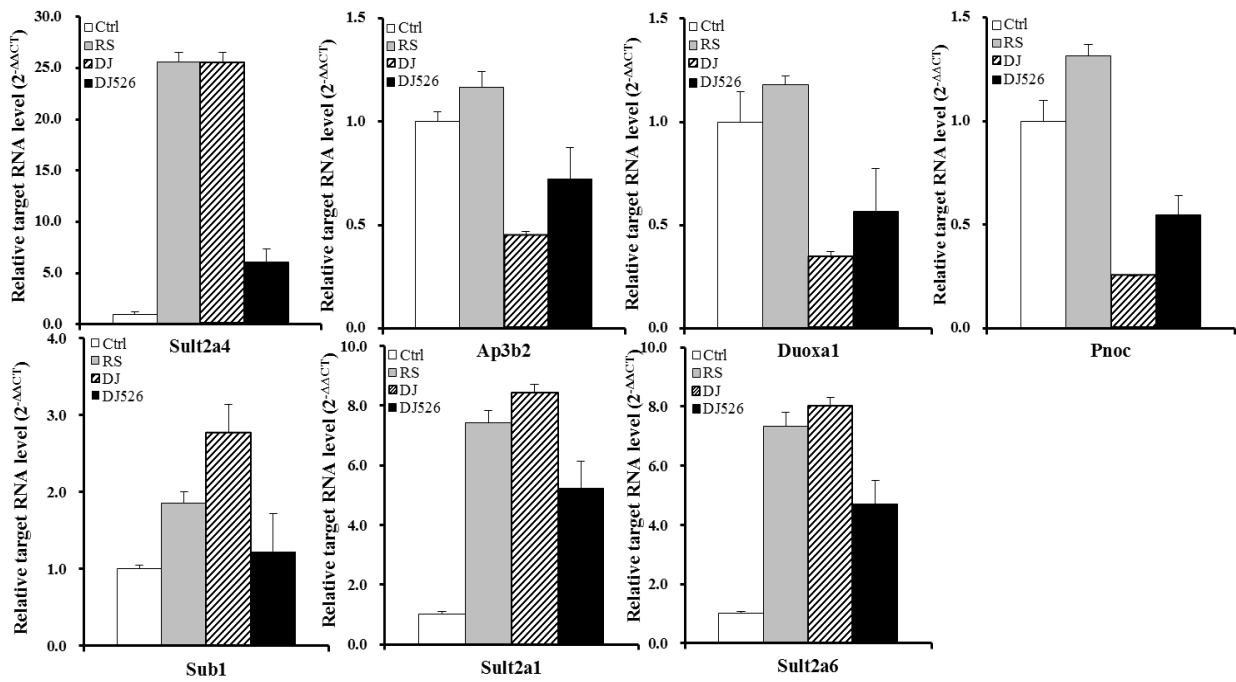


Supplementary Figure S1. The effects of the resveratrol-enriched rice DJ526 on changes in fat mass index with age-progression. The control (Ctrl) mice fed a NFD in which the carbohydrate source was corn starch and sucrose; the resveratrol (RS) mice fed a NFD in which the carbohydrate source was corn starch and sucrose except containing resveratrol; Dongjin (DJ) mice fed a NFD in which the corn starch and sucrose were replaced with Dongjin rice; DJ526, mice fed a NFD in which the corn starch and sucrose were replaced with the resveratrol enriched rice DJ526. The resveratrol concentration in DJ526 and RS groups was 0.69 $\mu\text{g/g}$ each, and not detected in Ctrl and DJ groups. Comparative analysis of the fat mass index (fat mass/total body weight) of mice at 0 month (a), 3 months (b), and at 6 months (c). The values represent the mean \pm s.d. (n=20). Values in the figure with a superscripted letter indicate statistical significance as analyzed by an unpaired Student's t-test; *p < 0.05 compared with Ctrl.



Supplementary Figure S2. The gene set enrichment analysis of the microarray data from the mice on resveratrol (RS), Dongjin rice (DJ), resveratrol-enriched rice DJ526 (DJ526), or control (Ctrl) diet. The control (Ctrl) mice fed a NFD in which the carbohydrate source was corn starch and sucrose; The resveratrol (RS) mice fed a NFD in which the carbohydrate source was corn starch and sucrose except containing resveratrol; Dongjin (DJ) mice fed a NFD in which the corn starch and sucrose were replaced with

Dongjin rice; DJ526, mice fed a NFD in which the corn starch and sucrose were replaced with the resveratrol enriched rice DJ526. The resveratrol concentration in DJ526 and RS groups was 0.69 $\mu\text{g/g}$ each, and not detected in Ctrl and DJ groups. Columns show every pathway significantly up-regulated (red) or down-regulated (green) by each treatment at the end of the 6 month feeding experiments.



Gene symbol	Relative target RNA level ($2^{-\Delta\Delta C_t}$)				Z ratio (Array)			Fold change (Array)		
	Ctrl	RS	DJ	DJ526	RS/Ctrl	DJ/Ctrl	DJ526/Ctrl	RS/Ctrl	DJ/Ctrl	DJ526/Ctrl
Sult2a4	1	25.61	25.52	6.1	11.07	7.99	6.70	15.86	18.48	10.60
Ap3b2	1	1.17	0.45	0.72	5.63	3.36	5.13	4.75	2.61	5.15
Duoxa1	1	1.18	0.34	0.57	-1.83	-5.88	-4.34	0.87	0.20	0.42
Pnoc	1	1.31	0.25	0.55	-4.27	-5.68	-4.18	0.49	0.23	0.29
Sub1	1	1.86	2.78	1.22	3.08	5.36	2.17	2.57	3.66	2.09
Sult2a1	1	7.44	8.42	5.22	9.13	6.46	5.15	9.49	10.99	5.95
Sult2a6	1	7.33	8.02	4.7	6.77	4.63	3.70	4.96	5.00	3.27

Supplementary Figure S3. Validation of the microarray results by quantitative real-time PCR from the mice on resveratrol (RS), Dongjin rice (DJ), resveratrol-enriched rice DJ526 (DJ526), or control (Ctrl) diet. Total RNAs from the liver samples of Ctrl, RS, DJ or DJ526 groups were amplified using the primers specific for the indicated targets. The targets were selected based on the Z scores obtained by the microarray analyses, which were provided for the purposes of comparison. Note that Z score numbers reflect statistical confidence and do not relate directly to fold-change.