

Supplemental Table S1. Anthropometric, hematological, hematochemical, molecular, oxidative stress parameters and smoking habits.

| Variable (unit of measurement) | (a) Young adults (22-50 y.o.) N=19, M=7, W=12 | (b) Adults (51-70 y.o.) N=28, M=14, W=14 | (c) Older adults (71-99 y.o.) N=20, M=13, W=7 | (d) Ultra- centenarians (100-111 y.o.) N=11, M=2, W=9 | P |
|--------------------------------------|---|--|---|---|------------------------|
| BMI | 21.60 (17.30-30.20) N=19, (22.49±3.57) M=7, (25.21±2.52) W=12, (20.90±3.14) | 27.20 (21.40-35.40) N=27, (27.29±3.32) M=14, (27.41±2.61) W=13, (27.15±4.05) | 29.00 (18.70-40.90) N=20, (28.84±5.59) M=13, (28.82±4.72) W=7, (28.86 ±7.37) | 22.30 (18.40-29.70) N=10, (22.95±3.09) M=2, (26.10±5.09) W=8, (22.16 ±2.25) | a vs b,c d vs b,c |
| Hematochemical parameter | | | | | |
| CRP (mg/dL, <5) | 0.63 (0.16-23.23) N=19, (2.67±5.54) M=7, (0.60±0.55) W=12, (3.88±6.76) | 1.68 (0.42-63.86) N=27, (4.32±11.97) M=14, (5.90±16.71) W=13, (2.61±1.41) | 1.10 (0.23-26.75) N=20 (3.19±5.88) M=13, (3.40±7.07) W=7, (2.79±3.03) | 1.92 (1.13-5.97) N=11, (2.62±1.57) M=2, (3.36±0.45) W=9, (2.45±1.70) | ns |
| Molecular test | | | | | |
| RTL | 1.19 (0.95-1.51) N=19, (1.20±1.74) M=7, (1.12±0.20) W=12, (1.25±0.15) | 1.05 (0.68-1.31) N=27 (0.99±0.16) M=14, (0.98±0.17) W=13, (1.00±0.14) | 0.69 (0.45-1.19) N=20, (0.74±0.19) M=13, (0.73±0.16) W=7, (0.76±0.25) | 0.73 (0.47-0.85) N=10, (0.70±0.12) M=2, (0.67±0.12) W=8, (0.71±0.13) | a vs b,c,d b vs c,d |
| Oxidative stress parameters | | | | | |
| PON (U/L) | 122.81 (40.63- 392.43) N=19, (149.63±91.62) M=7, (136.39±75.73) W=12, (157.36±102.13) | 91.42 (43.40-300.09) N=27, (112.41±62.84) M=14, (115.88±74.57) W=13, (108.67±50.04) | 100.65 (19.39- 222.53) N=19, (99.28±57.82) M=12, (106.49±62.23) W=7, (86.93±51.48) | 78.02 (43.40-254.85) N=10, (105.26±72.61) M=2, (137.58±96.63) W=8, (97.19±71.21) | ns |
| TEAC (mM) | 3601.24 (2589.58- 4721.66) N=19, (3577.83±463.34) | 3794.43 (1805.68- 5485.35) N=27, (3784.18±686.92) | 3574.19 (2819.23- 5572.38) N=19, (3764.02±718.84) | 3650.68 (2967.68- 4658.10) N=10, (3725.91±502.69) | ns |

| | | | | | |
|-----------------------|--|--|--|---|----------|
| | M=7, (3523.58±653.98) W=12, (3609.47±339.18) <u>2.61 (1.19-4.52)</u> N=19, (2.76±1.02) M=7, (3.23±0.84) W=12, (2.48±1.04) | M=14, (3837.36±839.79) W=13, (3726.92±501.50) <u>2.62 (0.95-4.45)</u> N=27, (2.67±1.03) M=14, (2.62±0.92) W=13, (2.71±1.17) | M=12, (3596.05±572.30) W=7, (4051.99±892.50) <u>2.84 (0.64-4.18)</u> N=19, (2.63±1.02) M=12, (2.98±0.95) W=7, (2.04±0.91) | M=2, (3812.89±1195.31) W=8, (3704.17±343.64) <u>2.55 (1.12-5.78)</u> N=10, (2.77±1.37) M=2, (2.55±0.25) W=8, (2.82±1.55) | ns |
| Smoking habits | | | | | |
| Smoker | N=5, 26.32% M=2, 28.57% W=3, 25.00% | N=7, 29.19% M=2, 18.18% W=5, 38.46% | N=3, 15.00% M=3, 23.08% W=0, 0% | N=0, 0% M=0, 0% W=0, 0% | |
| Ex-smoker | N=2, 10.53% M=0, 0% W=2, 16.67% | N=10, 41.67% M=6, 54.55% W=4, 30.77% | N=10, 50.0% M=8, 61.54% W=2, 28.57% | N=0, 0% M=0, 0% W=0, 0% | p=0.001* |
| Never smoked | N=12, 63.16% M=5, 71.43 W=7, 58.33% | N=7, 29.17% M=3, 27.27% W=4, 30.77% | N=7, 35.00% N=2, 15.38% W=5, 71.43% | N=11, 100% M=2, 100% W=9, 100% | |

Abbreviations: y.o.=years old; N=total number of cases; M=men; W=women; SD=standard deviation; BMI=body mass index; CRP=C-reactive protein; RTL=relative telomere length; PON=paraoxonase; TEAC=trolox equivalent antioxidant capacity; MDA=malondialdehyde.

Data underlined are the median (min-max) of the total number of cases. Data between round brackets are mean values ± SD.

a, b, c, and d indicate, respectively, young adults, adults, older adults, and LLIs.

The table shows the pairwise comparisons between the different groups, *i.e.*, a, b, c, d. p-value≤0.05 is considered significant; ns=not significant.

Bonferroni test is used for pairwise comparisons.

*To test if there is difference in the smoking habit for Age Class is used the Fisher's exact test.

Data are presented both as median, minimum, maximum values, and mean±standard deviation (SD). For continuous variables, the one-way Analysis of Variance (ANOVA) is used to test if age produces a significant effect on the parameters. The Fisher test is considered to evaluate the significance of the results. For each statistically significant effect, we conduct a post hoc multiple comparison test using Bonferroni method. For categorical variables, we consider the chi-squared test or the Fisher exact test to compare differences between groups of age. All analyses were performed using Stata version 16.1 and all hypothesis testing are considered statistically significant for p≤ 0.05.

Supplemental Table S2. KEGG pathway of miR-21-5p and miR-126-3p.

| KEGG pathway | Predicted miR-21-5p target genes | Predicted miR-126-3p target genes |
|--------------|----------------------------------|-----------------------------------|
|--------------|----------------------------------|-----------------------------------|

| | | | |
|--|---|---|---|
| hsa04022_cGMP-PKG_signaling_pathway | OPRD1 KCNMA1 EDNRA ATF2 CALML4 PIK3R3 SOD2 PIK3R3 PIK3R3 APH1B | ATP2B4 CALML4 ADCY1 MYLK3 ROCK2 INPP5K INPP5B DGKB ATF2 ADCY1 PPARGC1A HDAC2EIF4EBP DVL1 HDAC2 | CREB5 PDE5A MEF2D MEF2C DGKD IPPK DGKH PPIP5K2 CREB5 FOXO3 FOXO3 DTX4 DTX3L |
| hsa04070_Phosphatidylinositol_signaling_system | | | |
| hsa04211_Longevity_regulating_pathway | | | |
| hsa04213_Longevity_regulating_pathway | | | |
| hsa04330_Notch_signaling_pathwa | | | |

Top 5 enriched KEGG pathway clustered by validated targets of miR-21-5p and miR-126-3p and corresponding target genes.

Supplemental Table S3. REACTOME pathway of miR-21-5p and miR-126-3p.

| REACTOME pathway | Predicted miR-21-5p target genes | Predicted miR-126-3p target genes |
|---|--|---|
| R-HSA-9614085_FOXO-mediated transcription | CITED2 PPARGC1A SMAD4 SOD2 YWHAZ | RBL2 BCL6 FOXO3 |
| R-HSA-400253_Circadian Clock | ARNTL ATF2 CLOCK HDAC2 PPARGC1A | CREM CRTIC1 MEF2C MEF2D |
| R-HSA-9615017_FOXO-mediated transcription of oxidative stress | PPARGC1A | FOXO3 PLXNA4 |
| R-HSA-9013148_CDC42 GTPase cycle | ARHGAP31 GIT2 PAK3 SH3PXD2A | ARAP2 ARHGAP35 ARHGAP42 CDC42BPA FARP1 FNBP1L IQGAP1 MCF2L |
| R-HSA-449147_Signaling by Interleukins | BRWD1 HDAC2 ITGB2 PIK3R3 PPIA PSMD8 PSMF1 RAP1B SOD2 SRGAP1 STAT5A | OPRD1 PSD8 SOD2 STAT5A |

TOLLIPUBE2V1
WIPF2 YWHAZ

Top 5 enriched REACTOME pathway clustered by validated targets of miR-21-5p and miR-126-3p and corresponding target genes.

Supplemental Table S4. KEGG pathway of miR-146a-5p and miR-181a-5p.

| KEGG pathway | Predicted miR-146a-5p target genes | | | Predicted miR-181a-5p target genes | | |
|--|------------------------------------|--------|------------|------------------------------------|---------|--------|
| hsa05205_Proteoglycans_in_cancer | CBL | ERBB4 | FZD1 | ANK3 | CAMK2D | |
| | | FZD8 | HSPG2 | | CCND1 | |
| | | IHH | IQGAP1 | | CDKN1A | |
| | | | MAPK13 | | ESR1 | FZD5 |
| | | MSN | PIK3CA | | HPSE2 | ITPR2 |
| | | PLCE1 | PPP1R12B | | | PRKACB |
| | | | PRKACA | | RAC1 | TP53 |
| | | | PRKGCSMAD2 | | | WNT5A |
| | | | WNT16 | | | |
| hsa04020_Calcium_signaling_pathway | ERBB4 | GRIN2A | | ADORA2B | | |
| | | HTR2C | ITPKB | CACNA1B | | |
| | | MYLK3 | NOS1 | CALM1 | | |
| | | PHKA2 | PLCD3 | CAMK2D | | |
| | | PLCE1 | PRKACA | ITPR2 | Orai2 | |
| | | | PRKGCG | | PHKG2 | |
| | | | | | PRKACB | |
| hsa04070_Phosphatidylinositol_signaling_system | BPNT2 | CDS1 | INPP4B | | | |
| | | ITPKB | MTMR14 | CALM1 | ITPR2 | |
| | | | MTMR8 | | MTMR7 | |
| | | | PIK3CA | | PPIP5K1 | |
| | | PLCE1 | PRKGCG | | PTEN | |
| | | | SLC8A1 | | | |
| hsa04110_Cell_cycle | HDAC2 | ORC4 | RBX1 | ABL1 | CCND1 | CDC27 |
| | | | | | CDC6 | |
| | | | SMAD2 | | CDKN1A | |
| | | | SMC1A | | | |
| | | | TFDP2 | | MCM3 | SKP1 |

| | | | |
|-------------------------|---------|-------|---------|
| | | SKP2 | SMC1B |
| | | TP53 | |
| | | CALM1 | CAMK2D |
| | | CDC27 | CPEB3 |
| | MAPK13 | PGR | ITPR2 |
| hsa04114_Oocyte_meiosis | PPP2R5E | | PRKACB |
| | PRKACA | | RPS6KA6 |
| | RBX1 | SMC1A | SKP1 |
| | | | SMC1B |
| | | | SPDYE11 |
| | | | SPDYE17 |

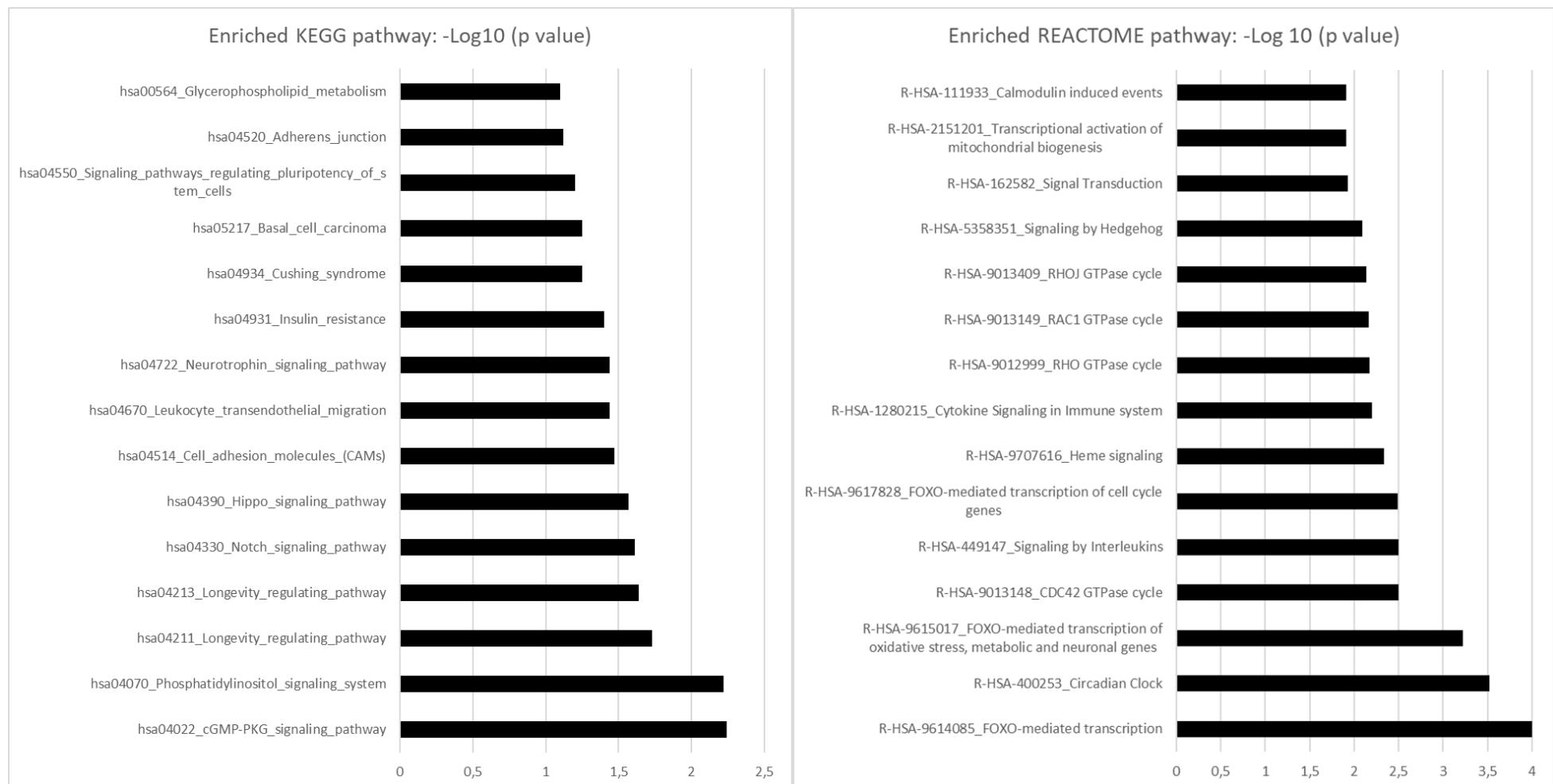
Top 5 enriched KEGG pathway clustered by validated targets of miR-146a-5p and miR-181a-5p and corresponding target genes.

Supplemental Table S5. REACTOME pathway of miR-146a-5p and miR-181a-5p.

| REACTOME pathway | Predicted miR-146a-5p target genes | | | Predicted miR-181a-5p target genes | | |
|--|------------------------------------|--------------|--------------|------------------------------------|-------------|-------------|
| R-HSA-4090294_SUMOylation of intracellular receptors | PGR | SUMO3UBE2I | | VDR | ESR1 | NR1I2 PPARA |
| | ARHGEF7 | CACNB2 | EPB41 | CACNA1B | CALM1CAMK2D | |
| | ERBB4 | GLUL | KCNQ5KCNS1 | CHRNA9 | GLRA2 | |
| | KCNS2 | LRRTM2 | NCALD | GNAI2 | GRIP2 | KCND3 |
| | NLGN3NPTN | PPFIA1 | PPFIBP2 | KCNJ6 | KCNK10, | |
| | PPM1E | PRKAB2 | PRKACA | LIN7C | LRFN2 | NTRK3 |
| | PRKAR2B | PRKGCGSLC1A2 | | PRKACB | RAC1 | |
| | SLC38A2 | SLC6A11 | | RPS6KA6 | SLC1A3 | |
| | SLC6A4 | SLITRK4 | | SLC1A7 | SLC6A1 | |
| | TSPOAP1 | GRIN2A | KCNA2 | SNAP25 | SYN2 | |
| | KCNK7DLG2 | LRRC49 | | SYT2 | GRIN2A | KCNA2 |
| | ARHGEF7 | CACNB2 | ERBB4 | CACNA1B | CALM1CAMK2D | |
| | GLUL | GRIN2A | NCALD | CHRNA9 | GLRA2 | |
| | | PPFIA1 | PPM1E PRKAB2 | | GNAI2 | GRIN2B |
| | | PRKACA | PRKAR2B DLG2 | | GRIP2 | KCNJ6 LIN7C |
| | | PRKGCGSLC1A2 | SLC38A2 | | NPTN | PRKACB |
| | | SLC6A11 | SLC6A4 | | RAC1 | RPS6KA6 |
| | | TSPOAP1 | | | SLC1A3 | SLC1A7 |
| R-HSA-112316_Neuronal System | | | | | | |
| R-HSA-112315_Transmission across Chemical Synapses | | | | | | |

| | | | |
|--|-------------------------|---------------------|--------|
| | | SLC6A1 | SNAP25 |
| | | SYN2 | DLG2 |
| R-HSA-3232118_SUMOylation of transcription factors | PIAS1 UBE2I SUMO3TFAP2A | FOXL2 PIAS2 TP53BP1 | |
| R-HSA-3215018_Processing and activation of SUMO | SUMO3UBE2I | SENP2 | |

Top 5 enriched REACTOME pathway clustered by validated targets of miR-146a-5p and miR-181a-5p and corresponding target genes.



Supplemental Figure S1. The top 15 enriched KEGG and REACTOME pathways from predicted target genes of miR-21-5p and miR-126-3p searched by miRWalk.



Supplemental Figure S2. The top 15 enriched KEGG and REACTOME pathways from predicted target genes of miR-146a-5p and miR-181a-5p searched by miRWalk.