SUPPLEMENTAL MATERIAL
Supplemental Methods

Astronaut samples

We studied the levels of cf-mtDNA in the blood plasma of 14 astronauts who flew short (~5-13-day) ISS missions between 1998 and 2001. Information regarding de-identified blood samples, including Shuttle Space mission code, the approximate average age of the crew members, and time spent in space, along with experimental strategy, are depicted graphically and in the table below.

| Sample ID # | Shuttle Mission Code | Approximate Age of The Crew | Time in Space |
|-------------|----------------------|-----------------------------|---------------|
| A1          | STS99                | 45.2 ± 3.9                  | 11d 5hr       |
| A2          | STS99                | 45.2 ± 3.1                  | 11d 5hr       |
| A3          | STS106               | 42.0 ± 4.9                  | 11d 19hr      |
| A4          | STS100               | 42.0 ± 4.1                  | 11d 21hr      |
| A5          | STS102               | 44.8 ± 6.3                  | 12d 21hr      |
| A6          | STS92                | 42.3 ± 4.0                  | 12d 19hr      |
| A7          | STS92                | 42.3 ± 4.0                  | 12d 19hr      |
| A8          | STS103               | 42.6 ± 5.5                  | 7d 23hr       |
| A9          | STS100               | 42.0 ± 4.1                  | 11d 21hr      |
| A10         | STS104               | 42.4 ± 3.1                  | 12d 18hr      |
| A11         | STS104               | 42.4 ± 3.1                  | 12d 18hr      |
| A12         | STS104               | 42.4 ± 3.1                  | 12d 18hr      |
| A13         | STS93                | 44.2 ± 3.9                  | 4d 22hr       |
| A14         | STS88                | 41.3 ± 4.1                  | 11d 16hr      |
Blood was sampled at three different time points: 10 days before launch (L-10), the day of landing (R-0), and 3 days after return (R+3). Pre- and post-flight samples were stored at -80°C until use.

**Nuclear and MT-DNA Measurements**

cf-mtDNA was isolated using the DNeasy Blood and Tissue kit from Qiagen according to the manufacturer's protocol (Qiagen, USA). Mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) abundance was measured by real-time quantitative PCR (qPCR) using an Applied Biosystems 7900 Sequence Detection System (Applied Biosystems, USA). Primer pairs specific for mtDNA (MT-CO1 and MT-CO3) were designed to quantify mtDNA abundance, and human beta-globin primers were used for normalizing nDNA. Forward and reverse primer sequences are depicted below.

| mt-DNA abundance | Gene symbol | Species | Forward primer (5'-3') | Reverse primer (5'-3') |
|------------------|-------------|---------|------------------------|------------------------|
| MT-CO1           | Human       | GCCCTCGTAGACCTAACCATCTTC | GTAAGTTACAATATGGAGATTATCC |
| MT-CO3           | Human       | ATGACCCACCAATCACGATG   | ATCACATGGCTAGGCGGAG     |
| GV1              | Human       | TTCTAGCAACCTCAACAGACA | TGTCTCCACATGCCCAGTTTCT  |
| ACTB             | Human       | CTGGAACGGTGAGGTGACA    | AAGGCCCTCTCGTAAAAATGCA  |

**Thrombin plasma preparation for exosome precipitation**

Exosomes were isolated from blood plasma samples of 3 astronauts (A9, A11, and A12) at L-10, R-0, R+3 using the ExoQuick Plasma prep and Exosome precipitation kit (Cat # EXOQ5TM, System Biosciences, CA, USA). In brief, 400 μl of plasma was mixed with thrombin and kept at room temperature (RT) for 5 minutes. Subsequently, samples were centrifuged at 10,000 rpm for 5 minutes. According to the manufacturer's protocol, the supernatant was collected into a new sterile microcentrifuge tube. Samples were then incubated with the exosome precipitation solution and refrigerated at 4°C for 30 minutes. After centrifugation at 1,500 x g for 30 minutes at 4°C, a beige-colored pellet was observed. Finally, the supernatant was aspirated, and the pellet was dissolved in 100 μl of sterile 1x PBS.

**Exosomal DNA isolation**

Exosomal DNA isolation was performed using the XCF Exosomal DNA isolation kit (Cat # XCF200S-1, System Biosciences, CA, USA). Isolated exosomes were dissolved in 1x PBS, and the final volume was adjusted to 500 μl. Next, the binding buffer was added to the PBS-dissolved
exosomes. Exosomes were centrifuged using a column provided by the manufacturer and further washed using the washing buffer. Exosomal DNA was eluted using the elution buffer, according to the manufacturer's protocol.

**Exosome antibody array**
The exosome antibody array was performed using the Exo-check exosome antibody arrays (Cat # EXORAY210A-8, System Biosciences, CA, USA). Briefly, isolated exosomes were quantified for protein using the BCA assay kit (Cat # 23225, Thermo scientific, IL, USA). We used 50 μg of protein to incubate with the labeling reagent at RT for 30 minutes. Excess labeling reagent was removed according to the manufacturer's protocol. Labeled exosomes were blocked using a blocking buffer, and the membrane was exposed with exosomes facing up at 4º C overnight. The next day, the membrane was washed for 5 minutes at RT. The membrane was then incubated with the detection buffer at RT for 30 minutes. Subsequently, washing was done with wash buffer three times for five minutes at RT and developed using the chemiluminescence detection system (Clarity Western ECL substrate, cat # 170-5060S, Bio-Rad, USA).

**Library preparation and small RNA sequencing**
RNA quality was assessed using an Agilent TapeStation (Agilent, Palo Alto, CA, USA), and RNA concentration was quantified by Qubit 4.0 spectrophotometer. The library for small RNA sequencing was prepared using the Smarter smRNA-seq kit for Illumina (Takara Bio Inc., USA). The quantity and quality of amplified libraries were evaluated using Qubit (Invitrogen, Carlsbad, CA, USA) and Agilent TapeStation high sensitivity D1000 Screen Tape. Small RNA-seq libraries were sequenced using single-end 75 base pairs (PE75) sequencing chemistry on NextSeq 500 instruments following the manufacturer's protocols (Illumina).

**Sequencing data analysis**
Raw Fastq files were trimmed using cutadapt and built-in Illumina adapters. The quality of trimmed reads was assessed with FastQC, which is freely available at https://www.bioinformatics.babraham.ac.uk/projects/fastqc. Reads were aligned to human genome reference build GRCh38/hg38 with STAR aligner allowing for reads multi-mapping to different parts of the genome. GENCODE release 38 was used for gene and transcript annotations.
Raw counts were normalized by library size and transformed to log2 with edgeR package. The proportion of cf-mt-DNA vs. cf-nDNA gene expression was calculated as a ratio of total mt-DNA read counts to nDNA read counts. Differential expression of mt-DNA genes was assessed using limma.

**Real-time quantitative reverse transcription PCR**

We also isolated total RNA from peripheral blood mononuclear cells (PBMCs) of six astronauts pre-flight (L-10) and at two time points post-flight (R-0 and R+3). The real-time polymerase chain reaction was performed using SYBR green (Power up SYBR green master mix, cat # A25742, Applied Biosystem, USA) and QuantStudio™ 3 real-time PCR systems as recommended by the manufacturer. We measured the expression of genes encoding inflammation (IL-6, IL-8, TNF-α, IL-1α, IL-β), oxidative stress (SOD1, SOD2, GPX1, NOX4, CAT1, NOXA1, SERPINE1, HMOX1, NOS2, NRF2, PRDX3, DUOX1, TMOD1, APOE) and DNA damage markers (OGG1, GADD153, GADD45a, PARP1, and DNAPK). Please note, out of 14 astronauts, buffy coats were available only for individuals A7, A8, and A12. Forward and reverse primer sequences are depicted below.

| Application | Gene symbol | Species | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|-------------|-------------|---------|------------------------|-----------------------|
| RT-qPCR     | APOE        | Human   | TGGTGGCTTGGGATTACCT    | AGGCCTCACTGCGGCTGT    |
|             | CAT1        | Human   | CTTCTTGTCCAGGATGTTTCCA | TACCTTTGGGGAGTATTTGGA |
|             | DNAPK       | Human   | CAGGAGACTCTGCCTGCTG    | AAACACGAAAGCTGCTCTC  |
|             | DUOX1       | Human   | AGAAATGCCAGCTGCCACT    | CGGCACATCTCAACCAACCA |
|             | GADD153     | Human   | CAGATGTGCTTTCCAGACTGATC| TGGATCTCTCTTCATTCCAGGA|
|             | GAPDH       | Human   | CGACCACCTTTGCAAGCTCA   | AGGGGAGATTCAGTTGGG   |
|             | GPX1        | Human   | AGGTACTACTTATCGGAAATGTCGC | TGGAGGAATTGCAATCTTCTGTT |
|             | HMOX1       | Human   | GGTGATGGCCCTTCCTGACC   | CTTCGCCGTCAGCTCTTCTC  |
|             | HSP60       | Human   | AGATGTAATTTTGGTGCAAGTCC | CACACCATTTGGTACTTTGGA |
|             | HSP70       | Human   | GTCTCTAAATGTTGCTCAATTGGAGC | CAACTGCACAATATCATAGCAAG  |
|             | HSP90       | Human   | CCCAGAGTGCGAATTACCCG   | CTTTTCCAGAGACAGTGAGTG |
|             | IL1A        | Human   | AAGAGACATGCTCCCTCCATGTA | CTTTGAGGTAAAGCTGTTTG |
|             | IL1B        | Human   | ATGATGCGCTTTACAGTGGCAATG | ATCTTCCAGCTTCTGCTGAG |
|             | IL6         | Human   | AACAAGTGACATCTGGAAGATG | ACTCTCAAGTTCTTGAGG |
|             | IL8         | Human   | TGCCAAGAGTCTAAGAACTTTA | AGCTTCCTCTTCAACAGAG |
|             | NOXA1       | Human   | AACCAGATGGCCAGTGCTTCAA | AGAGGAGCTGTTGGACACCTT |
|             | NOS2        | Human   | ACACGAGCTGATTTCCACCA   | GTCCCTTTGATGCTGCTC   |
|             | Nox4        | Human   | CTGTATAAAGGGCCAGGTATC  | TTATCCACATCTCTGTTCTCC |
DNA/RNA Oxidative Damage

DNA/RNA oxidative damage was measured in PBMCs isolated from 6 astronauts (L-10, R-0) and 5 astronauts (R+3) using the DNA/RNA Oxidative Damage (High Sensitivity) ELISA Kit (Cayman Chemical, USA). This competitive assay can be used to measure all three OxGua species: 8-hydroxyguanosine (8-OHG), 8-hydroxy-2'-deoxyguanosine (8-OHdG), and 8-hydroxyguanine. The antibody recognizes damaged nucleic acid species and binds to the goat polyclonal anti-mouse IgG previously attached to the well. The plate was washed to remove any unbound reagents, after which Ellman's Reagent was added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is inversely proportional to the amount of free 8-OHdG present in the well during the incubation.

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM) and were analyzed using a paired t-test for comparisons between means, or one-way ANOVA for repeated measures using the mixed-effects model followed by a Tukey post hoc test for multiple comparisons. Statistical analysis was performed using GraphPad Prism 6, version 6.07 (GraphPad Software, Inc., La Jolla, CA, USA). Differences were considered statistically significant at p < 0.05.
Table S1. List of differentially regulated genes.

| Gene name | A9 | Fold Change | A11 | A12 |
|-----------|----|-------------|-----|-----|
|           | L-10 | R-0 | R+3    | L-10 | R-0 | R+3    | L-10 | R-0 | R+3    |
| MT-TF     | 11.91 | 11.72 | 11.18 | 12.58 | 9.99 | 11.24 | 11.27 | 10.78 | 10.45 |
| MT-RNR1   | 14.77 | 14.59 | 14.76 | 14.59 | 14.94 | 14.63 | 14.41 | 14.82 | 14.9  |
| MT-TV     | 14.24 | 13.87 | 14.23 | 14.42 | 10.92 | 13.48 | 14.22 | 13.98 | 12.01 |
| MT-RNR2   | 19.57 | 19.61 | 19.45 | 19.42 | 17.79 | 17.77 | 19.46 | 19.7  | 18.02 |
| MT-TL1    | 13.18 | 12.98 | 12.93 | 13.52 | 11.22 | 12.43 | 13   | 12.77 | 11.19 |
| MT-ND1    | 12.99 | 13.23 | 13.28 | 12.07 | 13.47 | 13.64 | 12.76 | 13.6  | 13.34 |
| MT-TI     | 9.91  | 9.89  | 8.84  | 9.47  | 8.12  | 8.96  | 10.03 | 9.79  | 8.53  |
| MT-TQ     | 12.04 | 11.27 | 11.29 | 13.08 | 11.67 | 10.82 | 11.96 | 11.72 | 11.59 |
| MT-TM     | 10.59 | 11.18 | 11.23 | 11.9  | 10.78 | 11.22 | 10.8  | 11.13 | 11.01 |
| MT-NDD    | 14.8  | 15.23 | 14.89 | 14.89 | 15.33 | 15.21 | 16.32 | 15.57 | 15.37 |
| MT-TW     | 11    | 10.66 | 11.09 | 11.49 | 11.13 | 12.03 | 10.44 | 11.05 | 11.45 |
| MT-TA     | 11.96 | 10.21 | 11.19 | 11.75 | 10.27 | 11.55 | 10.85 | 11.22 | 9.74  |
| MT-TN     | 10.66 | 10.21 | 10.49 | 11.28 | 10.59 | 14.27 | 10.63 | 10.76 | 10.36 |
| MT-TC     | 11.44 | 11.01 | 11.22 | 11.12 | 10.08 | 11.1  | 11.18 | 10.97 | 10    |
| MT-TY     | 10.65 | 10.14 | 10.19 | 11.4  | 9.43  | 9.49  | 11.37 | 10.93 | 9.74  |
| MT-CO1    | 13.6  | 14.61 | 14.35 | 12.29 | 14.37 | 14.45 | 13.71 | 13.68 | 14.21 |
| MT-TS1    | 13.62 | 13.04 | 12.98 | 14.42 | 12.25 | 13.02 | 14.45 | 14.11 | 12.39 |
| MT-TD     | 12.12 | 11.42 | 12.3  | 12.36 | 9.45  | 11.56 | 12.06 | 11.58 | 10.11 |
| MT-CO2    | 11.94 | 12.76 | 12.64 | 10.44 | 13.4  | 13.75 | 11.91 | 12.5  | 13.16 |
| MT-TK     | 12.78 | 12.6  | 13.08 | 13.79 | 11.64 | 12.91 | 13.54 | 13.2  | 12.52 |
| MT-ATP8   | 12.75 | 13.64 | 14.36 | 11.74 | 14.62 | 13.36 | 12.64 | 12.74 | 14.27 |
| MT-ATP6   | 12.43 | 13.34 | 14.13 | 11.29 | 14.28 | 14.59 | 12.03 | 12.34 | 13.5  |
| MT-CO3    | 13.67 | 14.31 | 13.83 | 12.93 | 15.04 | 13.63 | 13.64 | 13.89 | 14.97 |
| MT-TG     | 15.62 | 15.23 | 15.01 | 15.93 | 11.37 | 12.41 | 15.77 | 15.42 | 12.21 |
| MT-NDD    | 12.03 | 12.99 | 13    | 10.39 | 12.27 | 12.49 | 12.35 | 12.6  | 12.17 |
| MT-TR     | 12.04 | 11.58 | 12.28 | 12.71 | 9.79  | 11.83 | 11.3  | 10.8  | 10.79 |
| MT-NDD4L  | 13.25 | 14    | 13.08 | 10.22 | 13.12 | 11.62 | 12.3  | 12.93 | 13.1  |
| MT-NDD4   | 13.83 | 14.68 | 14.54 | 12.85 | 14    | 14.18 | 13.71 | 15.36 | 13.94 |
| MT-TH     | 11.46 | 11.17 | 12.18 | 12.16 | 10.74 | 12.38 | 11.64 | 11.49 | 10.98 |
| MT-TS2    | 10.68 | 10.55 | 10.91 | 12.64 | 9.35  | 11.52 | 11.61 | 11.21 | 10.38 |
| MT-TL2    | 14.7  | 13.76 | 13.66 | 15.15 | 10.37 | 12.21 | 14.09 | 13.94 | 11.5  |
| MT-NDD5   | 14.56 | 15.61 | 15.55 | 13.03 | 15.18 | 15.13 | 15.47 | 14.81 | 15.18 |
| MT-NDD6   | 11.94 | 13.12 | 13.08 | 10.28 | 12.98 | 12.83 | 12.04 | 15.26 | 12.67 |
| MT-TE     | 11.37 | 11.48 | 11.15 | 12.56 | 10.48 | 12    | 12.06 | 11.91 | 11.08 |
| MT-CYB    | 13.31 | 14.05 | 14.01 | 11.87 | 13.62 | 13.59 | 13.42 | 13.42 | 13.5  |
| MT-TT     | 11.8  | 12.06 | 12.17 | 12.13 | 10.21 | 11.33 | 12.24 | 11.8  | 10.39 |
| MT-TP     | 11.96 | 11.72 | 11.72 | 12.34 | 10.24 | 11.71 | 12.24 | 12.14 | 11.27 |

Gene expression of mt-DNA-encoded genes between control (L-10) and post-spaceflight (R0, R+3). Data are shown as fold change.
Figure S1. Comparison of cf-mtDNA and transcript levels of stress markers in PBMCs from three astronauts.

(A) Levels of cf-mtDNA at R0 and R+3 days in A7, A8, and A12 individuals. (B) Transcript levels of inflammatory markers (IL-1α, IL-1β, TNFα), and DNA damage markers (OGG1) follow similar trends to cf-mtDNA in 3 individual astronauts (light green background), whereas IL-6, IL8, SOD1, SOD2, GPX1, NOX4, GADD45, CAT1, DNA-PK, and PARP1 in at least one of the post-flight time points (light blue background).