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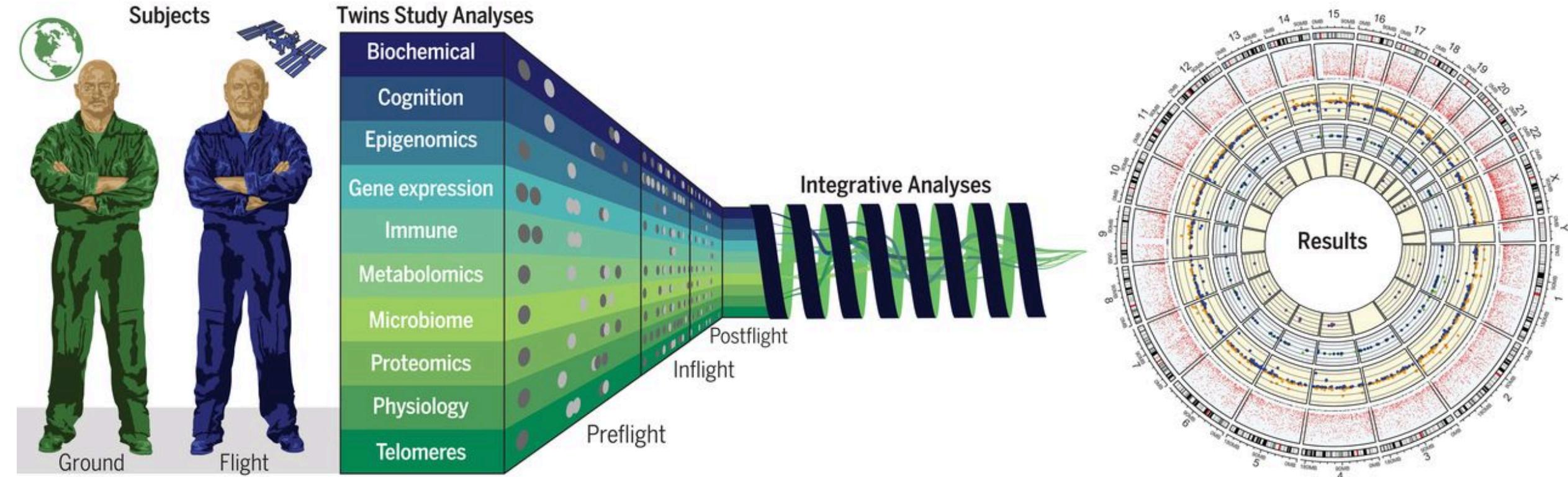
# The NASA Twins Study: A multidimensional analysis of a year-long human spaceflight

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Genomics Journal Club

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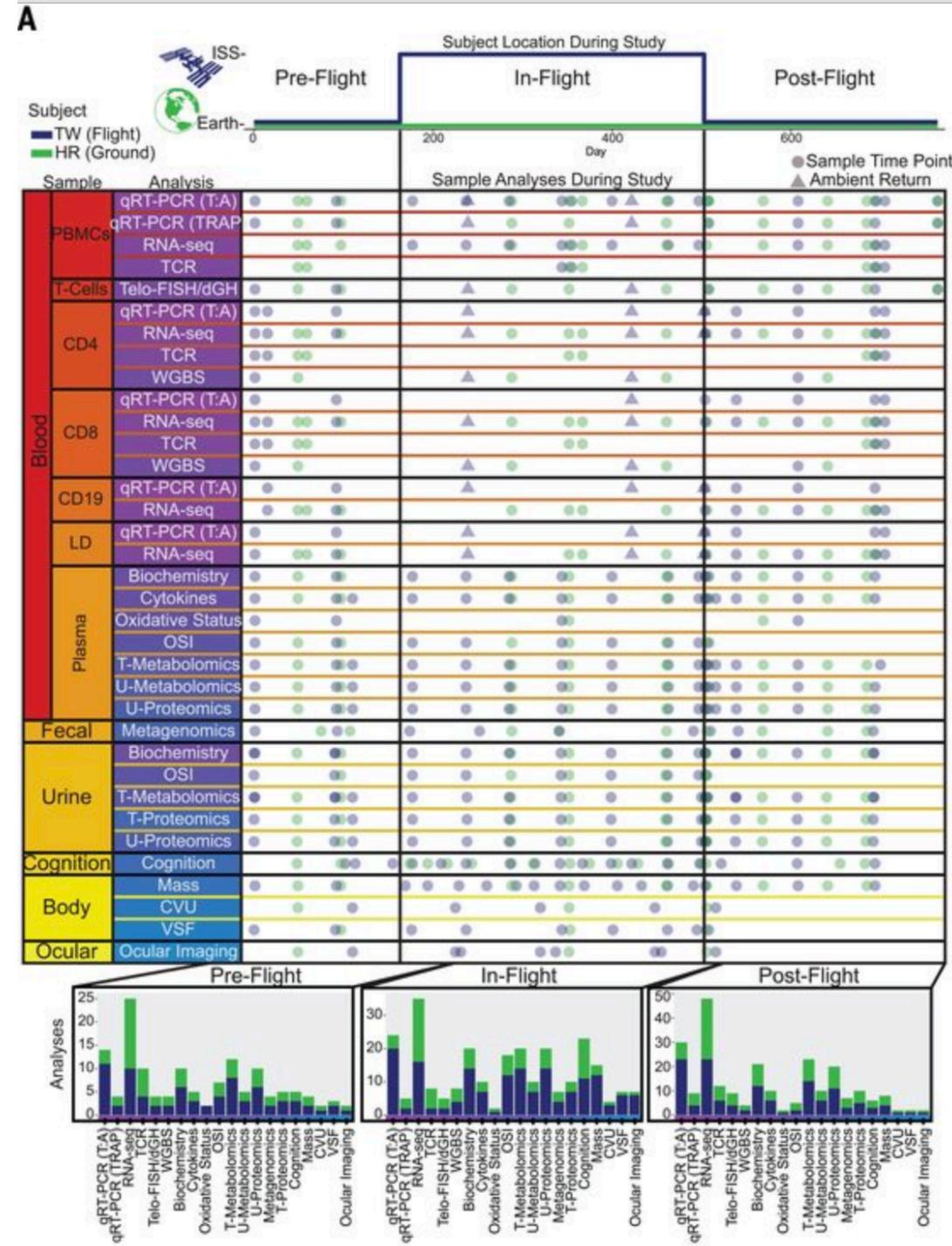
# What is the impact of long-duration space missions on human physiology?



Structured Abstract: Genetically identical twin subjects (ground [HR] and flight [TW]) were characterized across 10 generalized biomedical modalities before (preflight), during (inflight), and after flight (postflight) for a total of 25 months.

This is a massive study!  
Let's focus on the genomic assays.

1. Gene expression (RNA-seq)
2. Telomere length (qPCR and FISH)
3. Fecal metagenome
4. DNA methylation (WGBS)
5. Translocation/inversion  
(directional genomic hybridization dGH paints)



# Differentially expressed genes: DESeq2

B

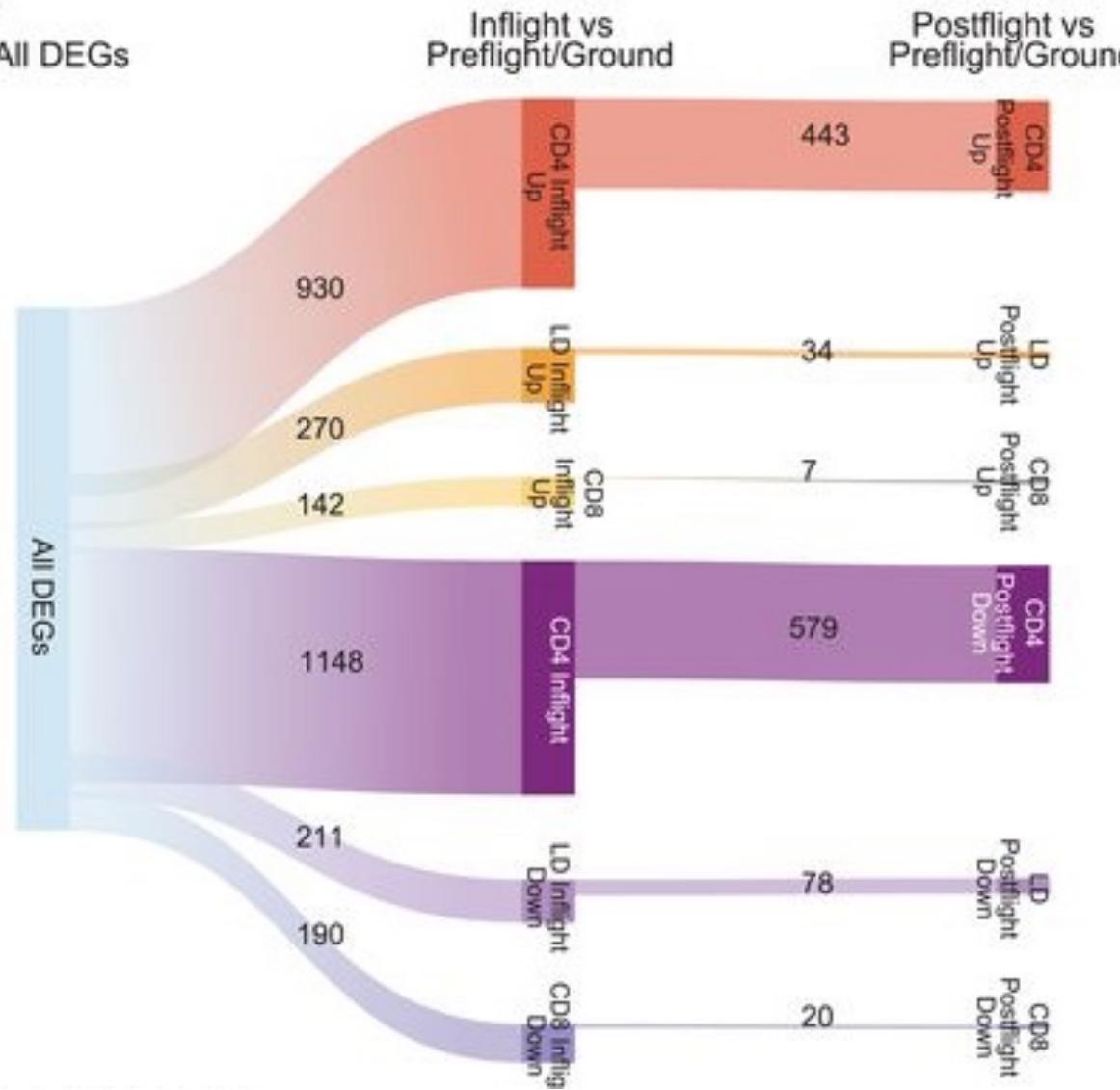
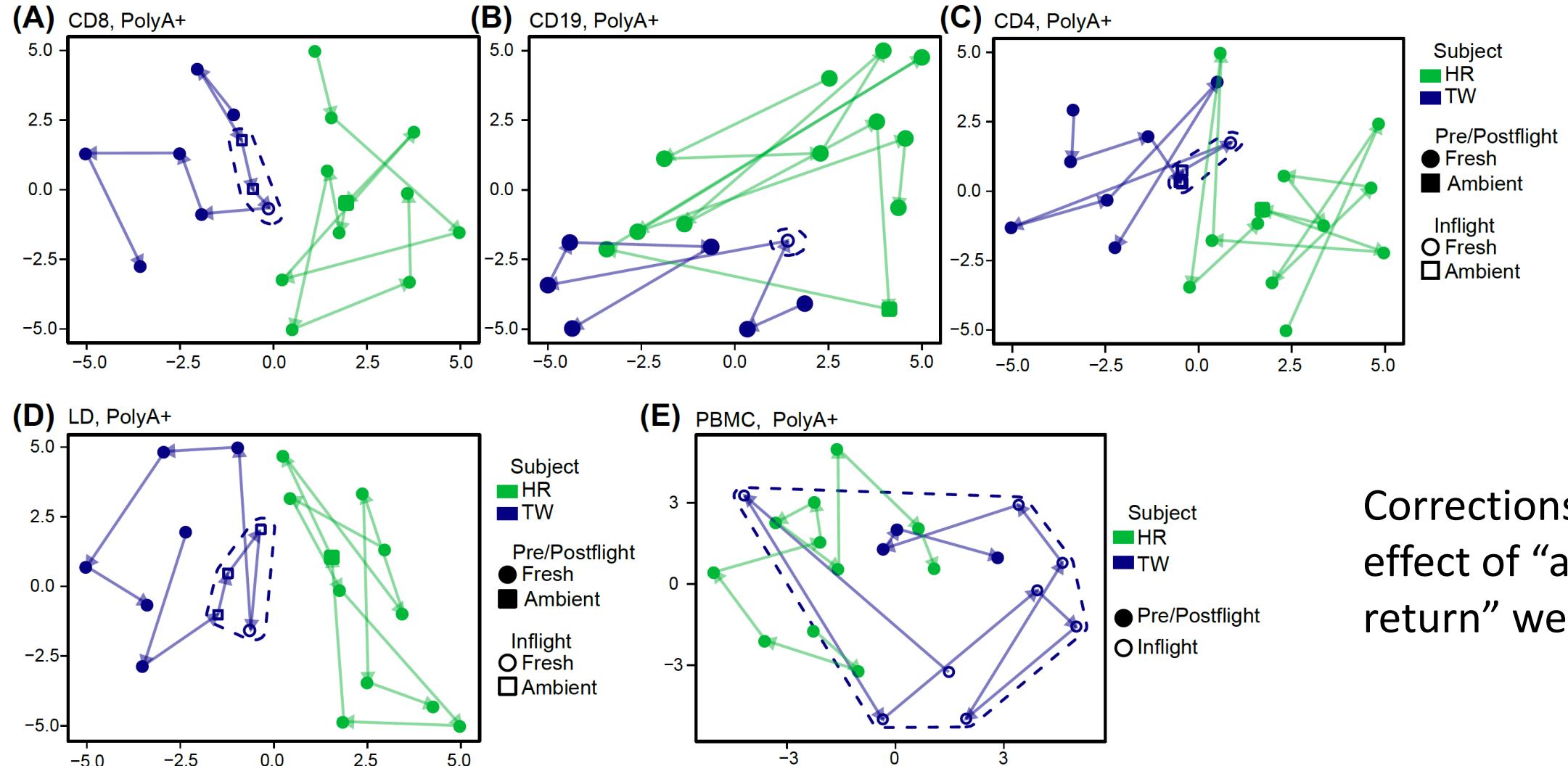


Figure 1B. Gene expression changes in TW inflight and postflight compared with the preflight period. All time points of HR were used to account for normal levels of variance and noise in gene expression. Genes that were significantly altered inflight after controlling for subject baselines and AR effect are reported. Gene expression changes were reported for any gene with  $q < 0.05$  in a multivariate model that utilized expression values for polyadenylated [poly (A)<sup>+</sup>] and ribosomal RNA depleted transcripts.

# tSNE with a “time axis” shows the changes in gene expression in different blood cell types



# Function of the differentially expressed genes

“Differential gene expression analysis indicated that many immune-related pathways were significantly changed inflight across all cell types (GLM,  $q < 0.001$ ), including the **adaptive immune system, innate immune response, and natural killer cell-mediated immunity** (table S2).”

“TW exhibited altered gene expression patterns in pathways related to **oxygen and reactive species metabolic processes, mitochondrial transport, hypoxia, and apoptotic mitochondrial changes** (GSEA,  $q < 0.0001$ ) (table S2).”

“[...] when inflight, TW’s down-regulated DEGs were significantly enriched in **packaging-of-telomere ends and telomere-maintenance pathways**, in both CD4 and CD8 cells [gene set enrichment analysis (GSEA), all  $q \leq 0.001$ ] (table S2).”

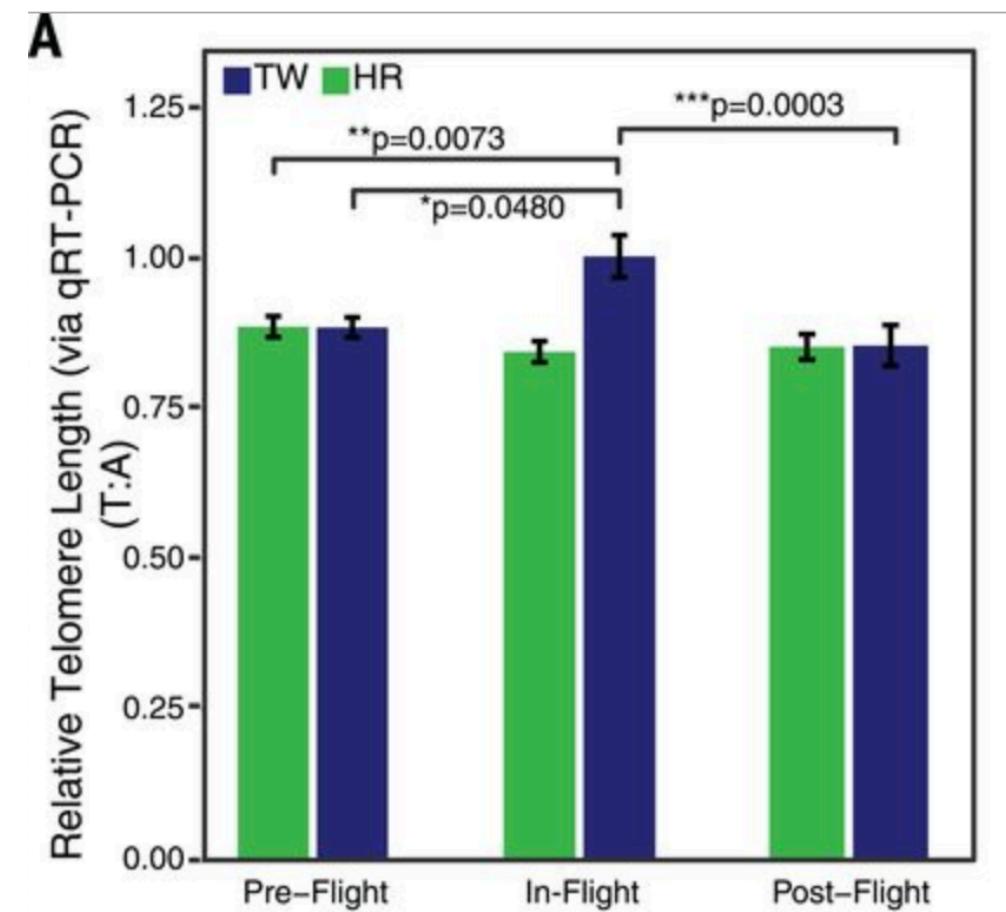
“[...] a distinct subset of genes did not return to preflight levels within 6 months of return to Earth (811 genes across different cell types), including genes related to **immune function and DNA repair**, representing candidate genes that may be altered for an extended period of time as a result of long-term spaceflight exposure.”

“The time spent on the ISS was correlated [coefficient of determination ( $R^2$ ) = 0.84] with increased **mtRNA** inflight ( $P = 3.6 \times 10^{-6}$ , ANOVA).”

# Telomere length change is significant!

- Started out very similar length, and ground subject's stayed the same
- 14.5% increase in telomere length in flight subject from day 14 on
- Telomeres shortened immediately upon return to ground and returned to "near-preflight averages" within months

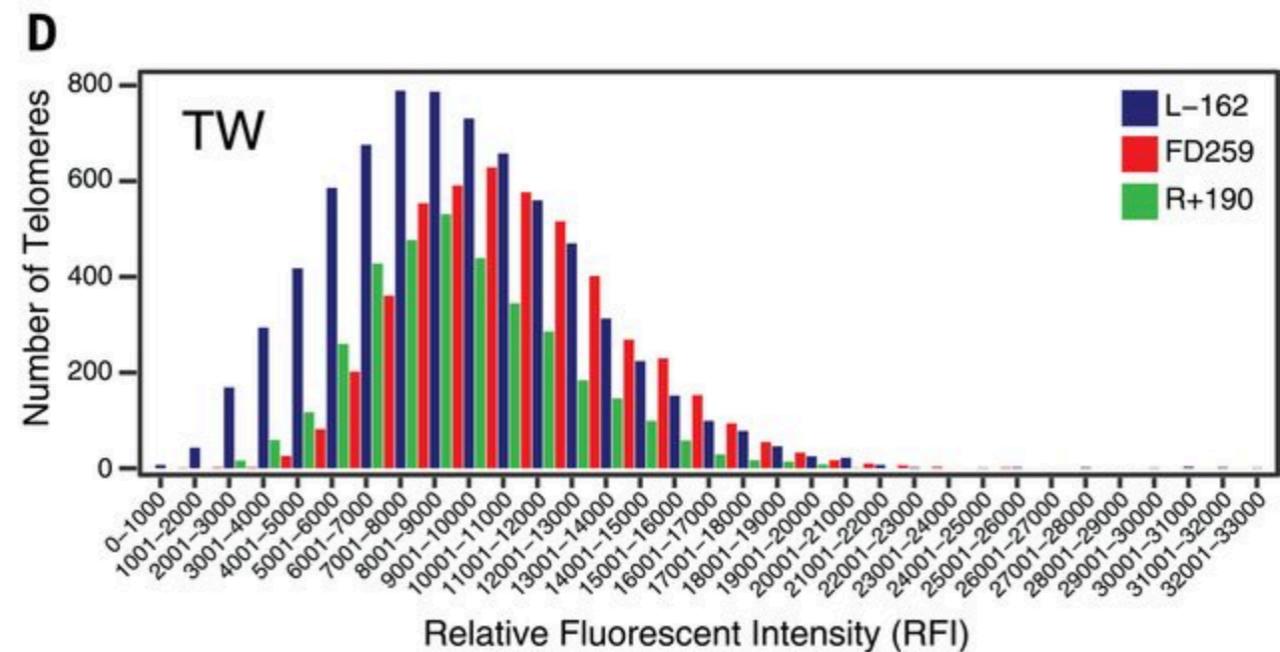
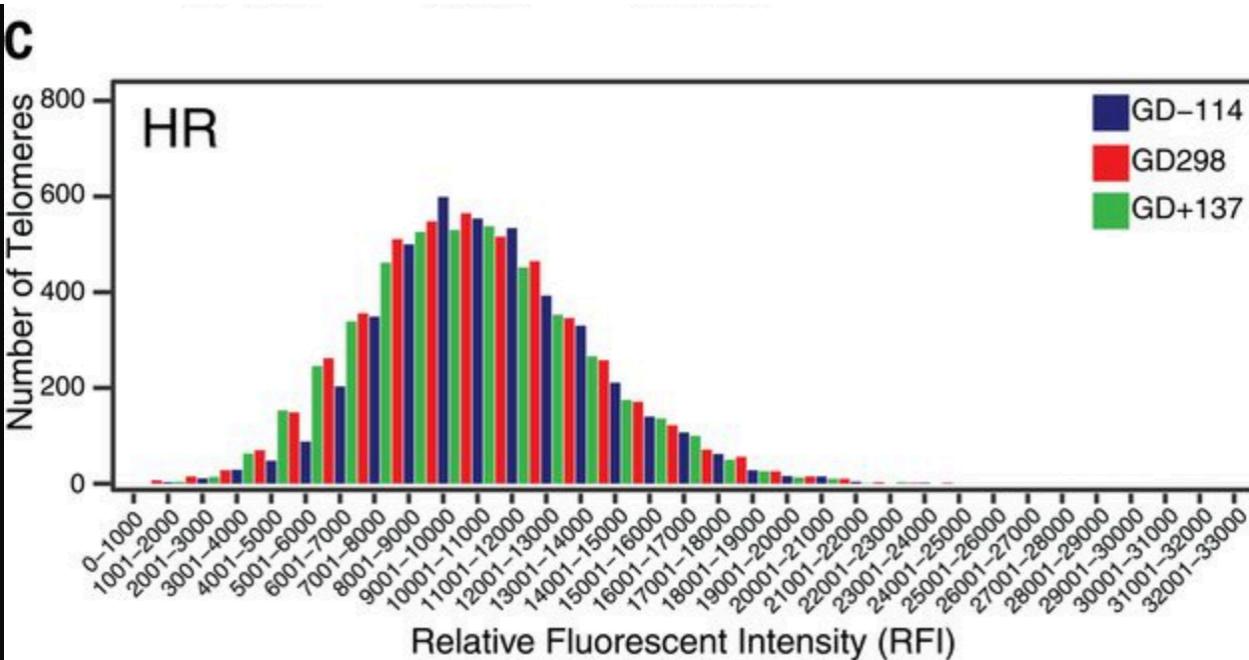
Figure 2A. Relative average telomere length in PBMCs (DNA) pre-, in-, and postflight assessed by qRT-PCR for HR (green) and TW (blue). Significance was tested by one-way ANOVA, and error bars represent SEM.



# Some telomeres “disappeared” postflight

Figure 2 C,D. Telo-FISH-generated histograms of individual telomere length distributions [shorter to longer, lower to higher relative fluorescent intensity (RFI)] for HR (C) and TW (D) preflight (blue), inflight (red), and postflight (green).

“However, an increased number of signal-free chromosome ends, indicative of complete loss and/or critically short telomeres (i.e., below our level of resolution), was also apparent”



# Telomere length change is not significant?

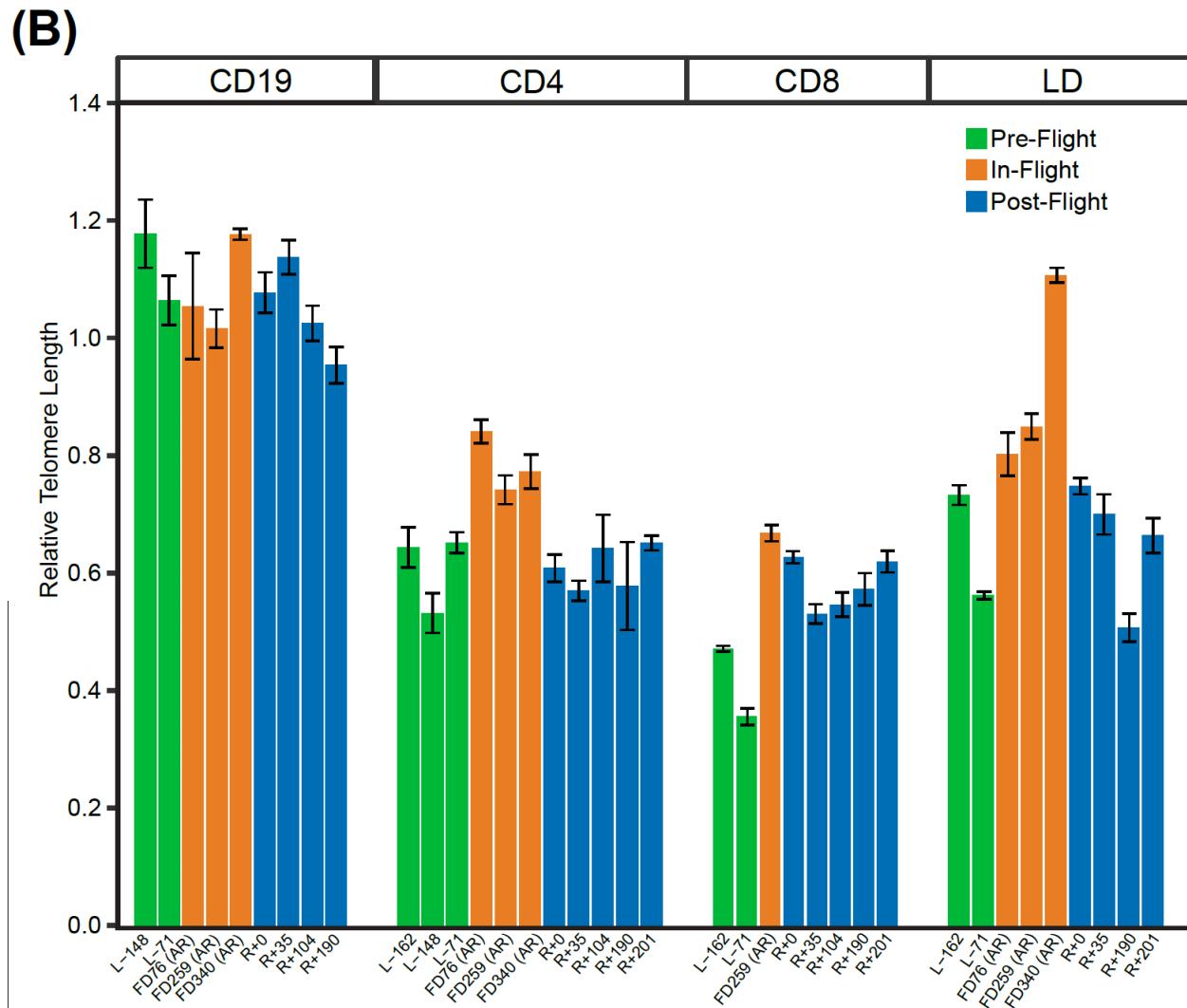


Figure S6. Telo-FISH cell-by-cell analysis of average telomere length (relative fluorescence intensity; RFI) on metaphase chromosomes (PHA stimulated T-cells) pre-, in-, and postflight; inflight differences were not statistically significant (one-way ANOVA). Error bars represent SEM.

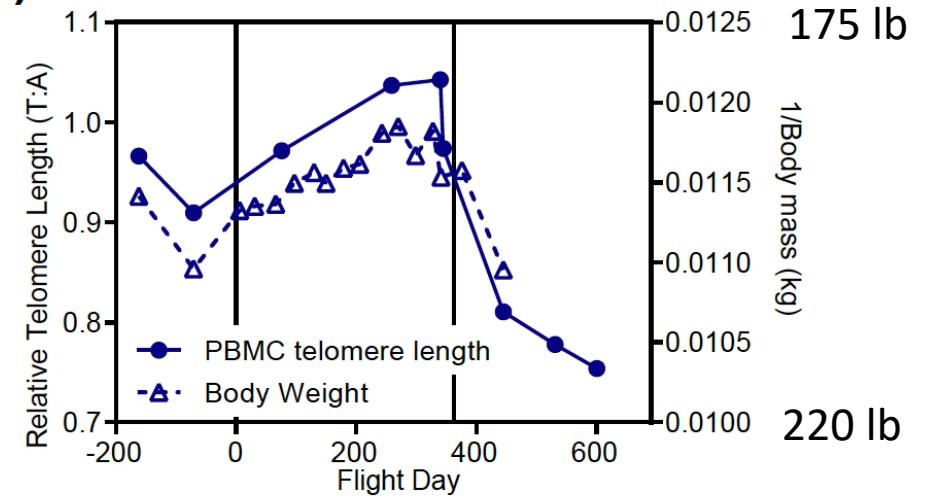
# Why the telomere change?

“Although the underlying mechanisms and potential consequences of such transient telomere elongation are currently unknown, healthy lifestyle factors related to **metabolic and nutritional status, physical activity, and weight loss** have been associated with longer telomeres. Consistent with these studies, TW’s reduced body mass and increased serum folate levels inflight correlated with telomere lengthening (fig. S15).”

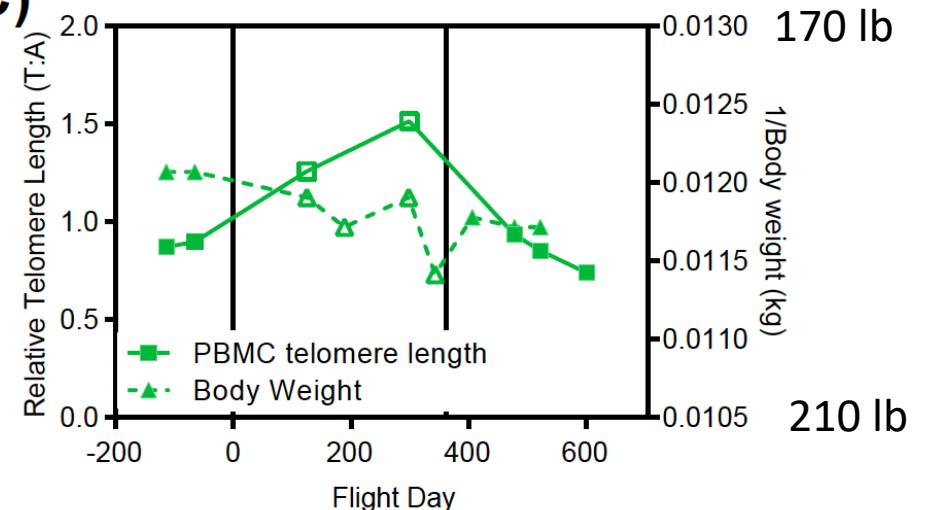
“TW lost 7% of his body mass during flight, whereas HR gained ~4% of his body mass over the course of the study.”

Figure S15

(A)

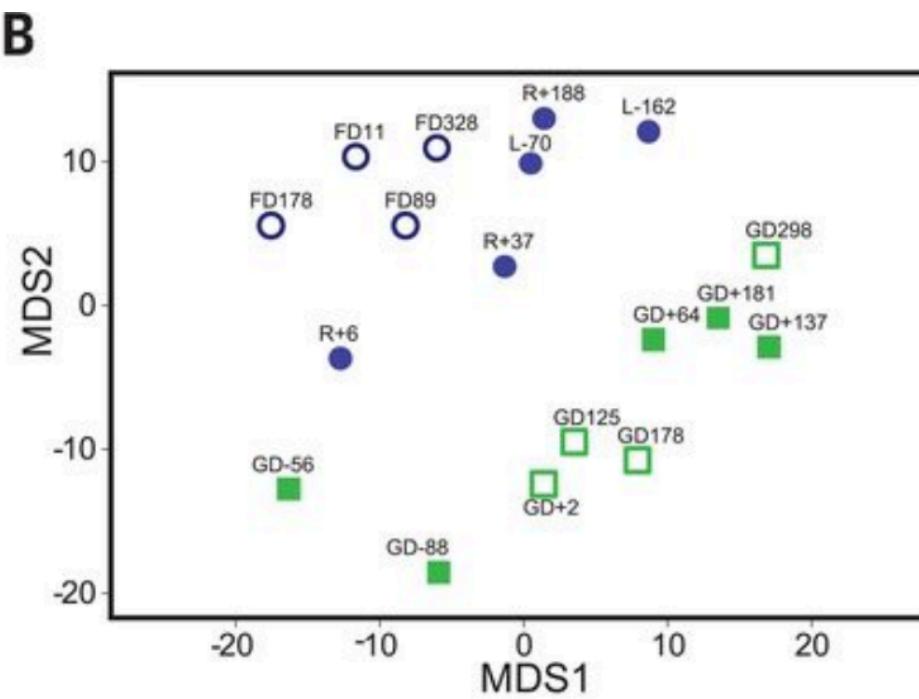


(C)

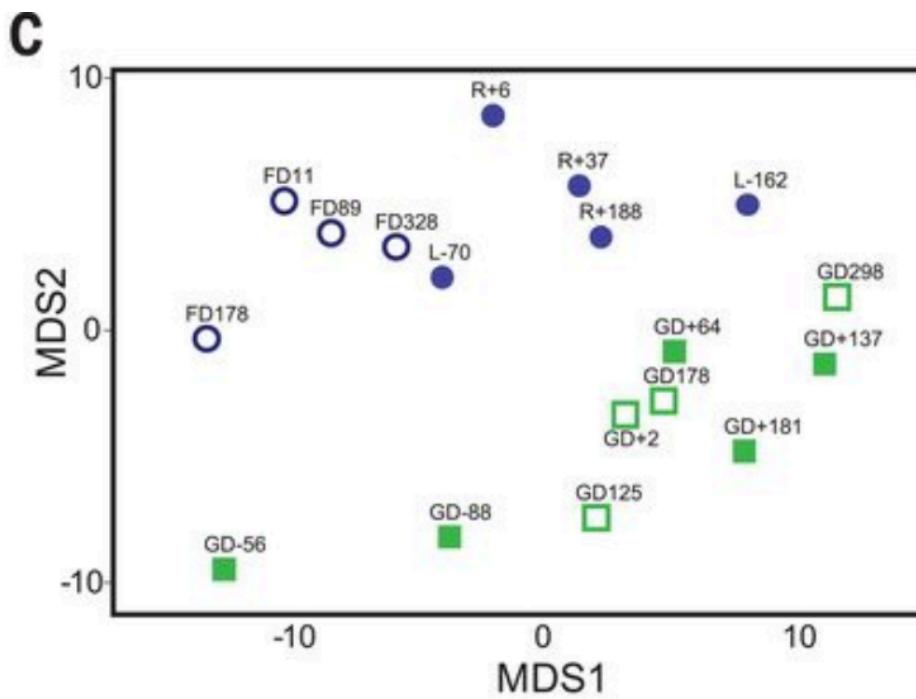


# Microbiome depends more on who you are than whether you spend a year in space

Genus



Functional

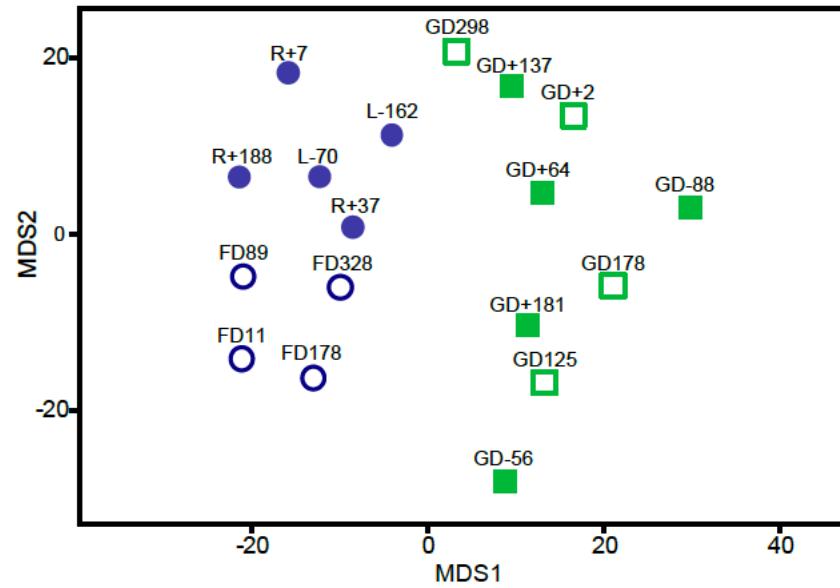


Analysis of microbial community taxonomic structure at the genus level (B) and functional gene content (C) using mMDS of annotated shotgun metagenome sequence data. Data were  $\log(x + 1)$  transformed, and a resemblance matrix, from Bray-Curtis dissimilarity, was generated. MDS axes 1 and 2 are plotted; two-dimensional stress values are 0.19 and 0.13 for taxonomic and functional gene analyses, respectively.

# Microbiome depends more on who you are than whether you spend a year in space

**Fungi** In flight subject, pre/post and inflight are significantly different

(A)



**Viruses** In flight subject, pre/post and inflight are NOT different

(B)

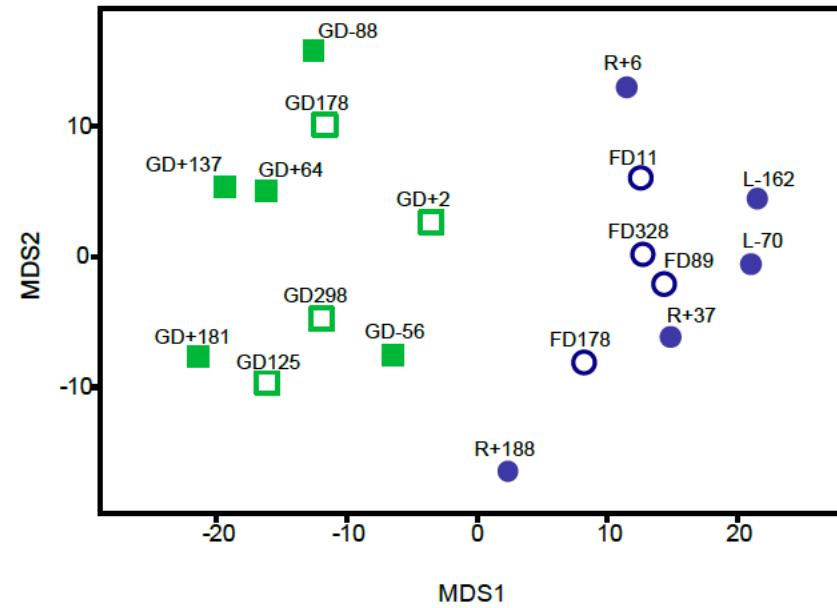


Figure S10. A. Metric multidimensional (mMDS) plot of fecal fungal microbial community from TW and HR. Samples are color coded by subject (TW=blue; HR=green), with open symbols representing flight samples from TW or flight equivalentsamples from HR.

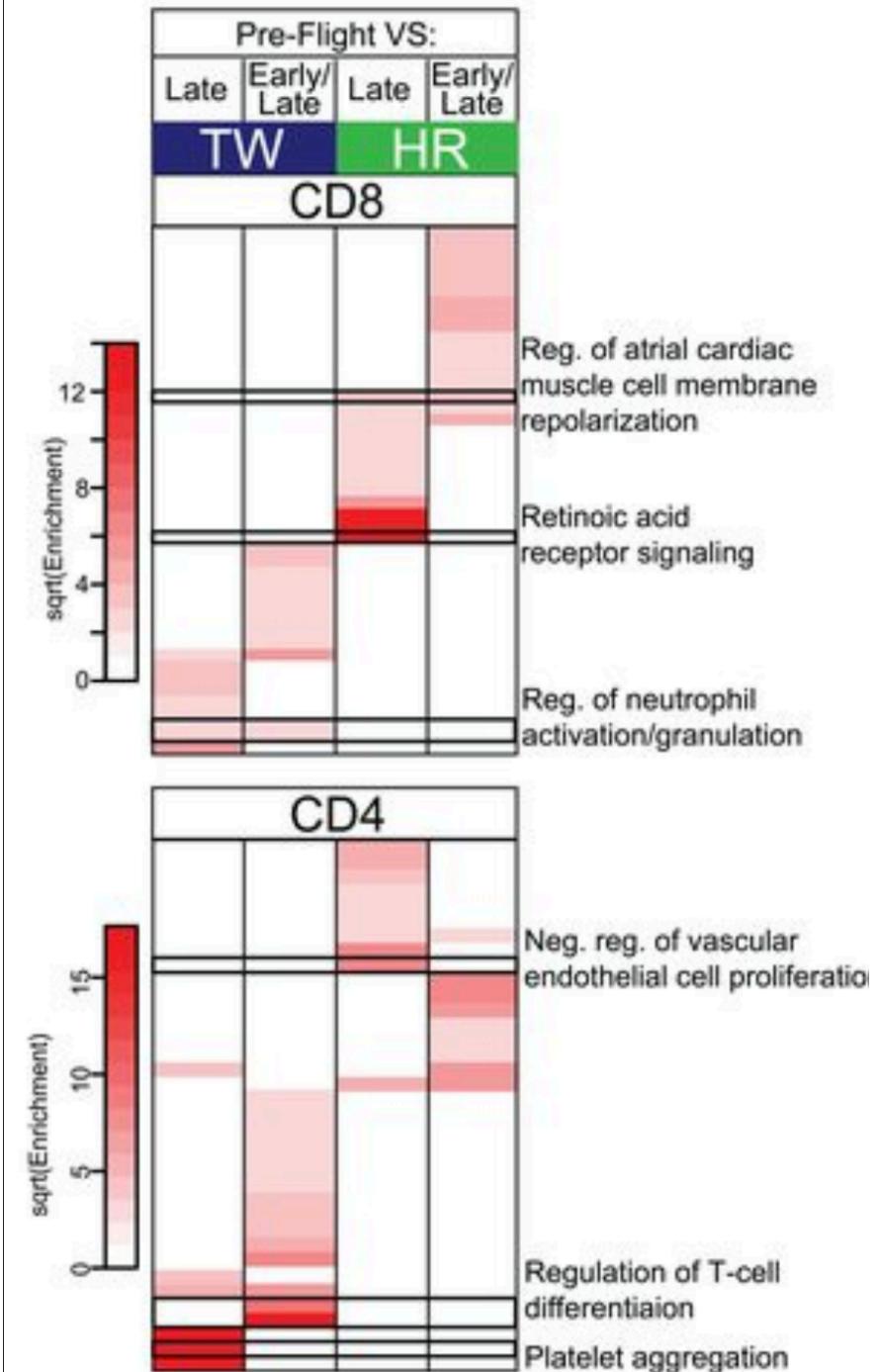
# Epigenetically, not much happened

“[...] although some epigenetic loci were changed, the genome-wide epigenetic variance was higher in the ground control than in the flight subject during the 25 months of this study.”

“DNA methylation analysis revealed epigenetic discordance (indicated by JSD peaks) for TW in the promoters of *NOTCH3*, a well-known regulator of T cell differentiation, and *SLC1A5/ASCT2*, which promotes T cell activation, between preflight (L-162) and inflight samples (FD259 for *NOTCH3* and FD76 for *SLC1A5*) in CD4 and CD8 cells, respectively”

# Function of the differentially methylated genes

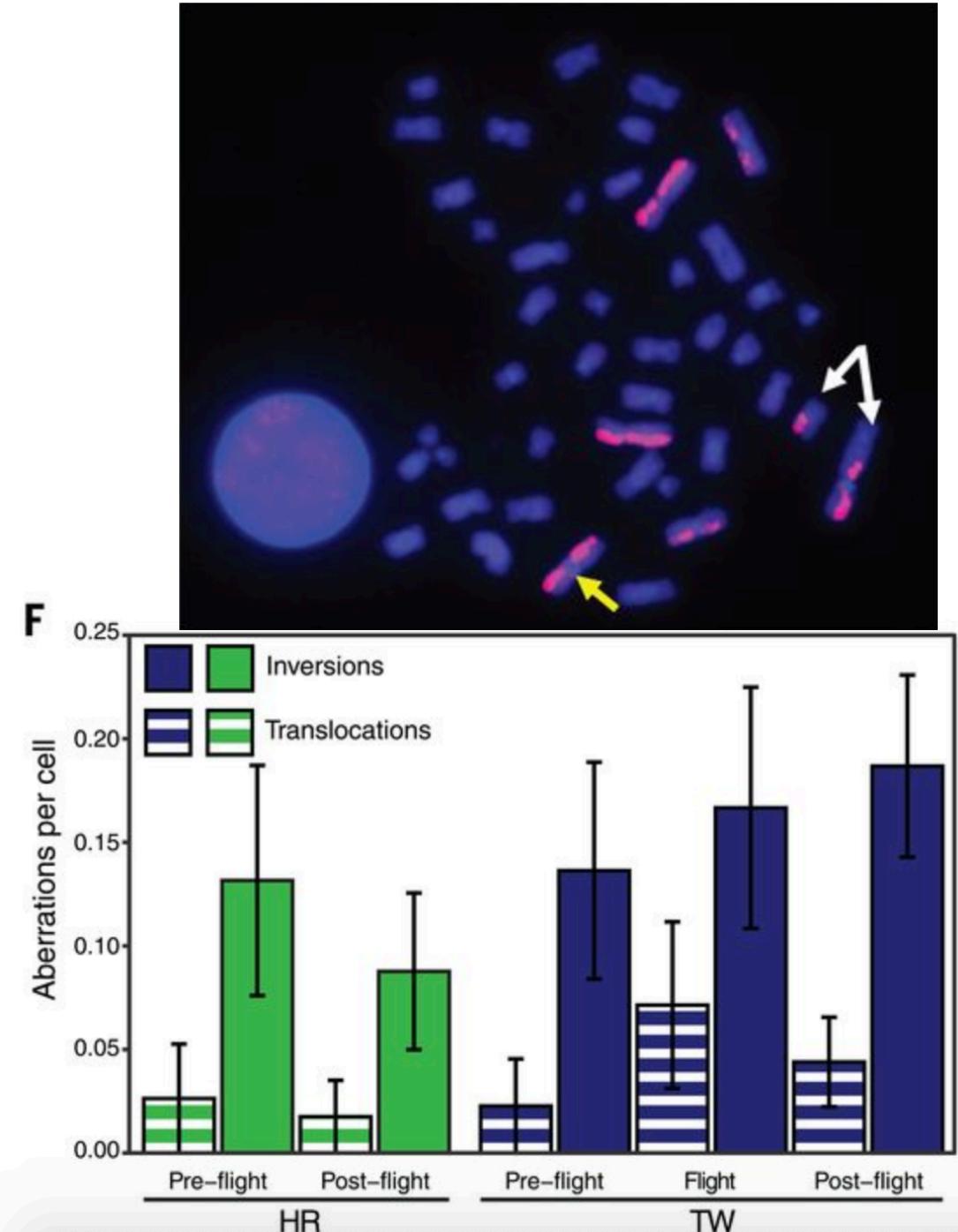
Figure 1D. GO analysis of genes ranked on the basis of epigenetic discordance at their promoters. Comparisons of preflight samples to inflight (early and late, and combined early and late) and postflight samples are shown for both CD4 and CD8 cells. Heatmaps represent transformed enrichment values [square root (sqrt) of enrichment] for GO categories with a raw enrichment value  $>5$ . Reg., regulation; neg. reg., negative regulation.



# “Chromosome Aberrations”

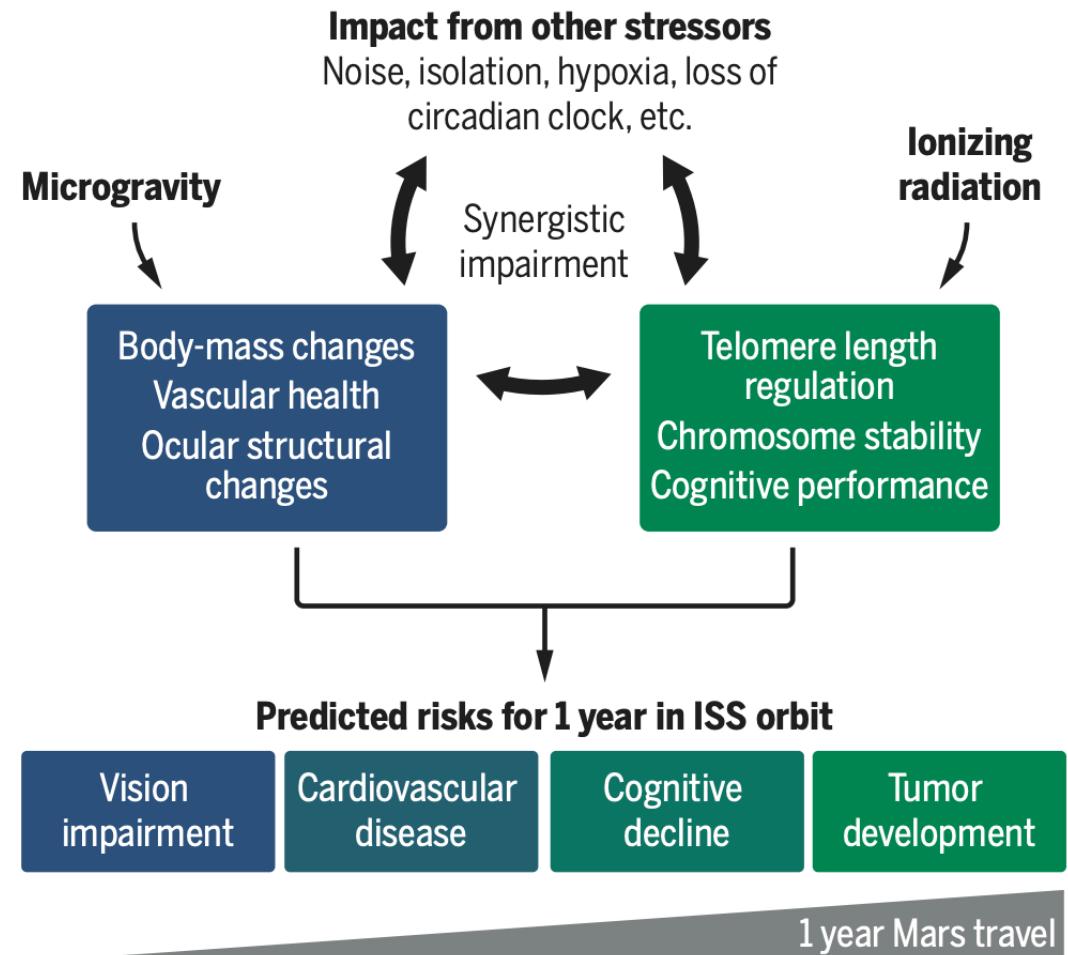
Figure 2E. Cytogenetic analysis of DNA damage utilizing dGH paints (pink) for chromosomes 1, 2, and 3 facilitated simultaneous detection of translocations and inversions. Representative image of dGH on a metaphase chromosome spread illustrating an intrachromosomal inversion (yellow arrow) and an interchromosomal reciprocal translocation (white arrows).

Figure 2F. Quantification of translocation (striped bars) and inversion (solid bars) frequencies for HR and TW pre-, in-, and postflight. Results were not statistically significant (one-way ANOVA). Error bars represent SEM.



# A year in orbit

A 1-year mission aboard the International Space Station (ISS) caused multiple alterations that arose from microgravity, ionizing radiation, and other stressors. These changes likely impose health risks.



The impact of ionizing radiation will be five times greater for Mars travel.

"Hazards of human spaceflight." Löbrich and Jeggo, *Science* (2019)



<https://www.nasa.gov/feature/nasa-releases-omics-advancing-personalized-medicine-from-space-to-earth-video>

# Some Interesting Tidbits

# Confounding factors of blood sample collection

- Blood collected from the flight subject was returned to Earth in a Soyuz capsule
- Similar, non-ideal environmental conditions were imposed on a control sample

(Supplementary Materials & Methods) “Blood was collected from a male volunteer of similar age and ethnicity to the study subjects [...] To simulate temperature and storage conditions during ambient return, AR controls were shipped at 4°C via Fedex for 3000 flight miles, re-packed and shipped again for 3000 flight miles, before being processed in the same manner as the FR control.”



# Response to radiation

“[...] the encountered radiation dose of **146 millisieverts** (mSv) (a measure for the biological effectiveness of radiation), which is equivalent to 50 years of natural background radiation on Earth.” (Löbrich and Jeggo, 2019)

In a **CT scan**, the organ being studied typically receives a radiation dose of **15 mSv** in an adult to 30 mSv in a newborn infant. A typical **chest X-ray** involves exposure of about **0.02 mSv**, while a dental one can be 0.01 mSv. (Reuters, 2011)

Exposure to **100 mSv a year** is the lowest level at which any increase in cancer risk is clearly evident. A **cumulative 1,000 mSv** (1 sievert) would probably cause a fatal cancer many years later in five out of every 100 persons exposed to it. [...] A single dose of **5 sieverts** would kill about half of those exposed to it within a month. (Reuters, 2011)

<https://www.reuters.com/article/us-japan-quake-radiation/factbox-how-much-radiation-is-dangerous-idUSTRE72E14R20110315>

If there were astronaut triplets, what experience should the 3<sup>rd</sup> sibling undergo?

What other events would it be interesting to study the physiological effects using in-depth analysis of twins?