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Supplemental Information

Circulating miRNA Spaceflight Signature Reveals Targets for Countermeasure Development

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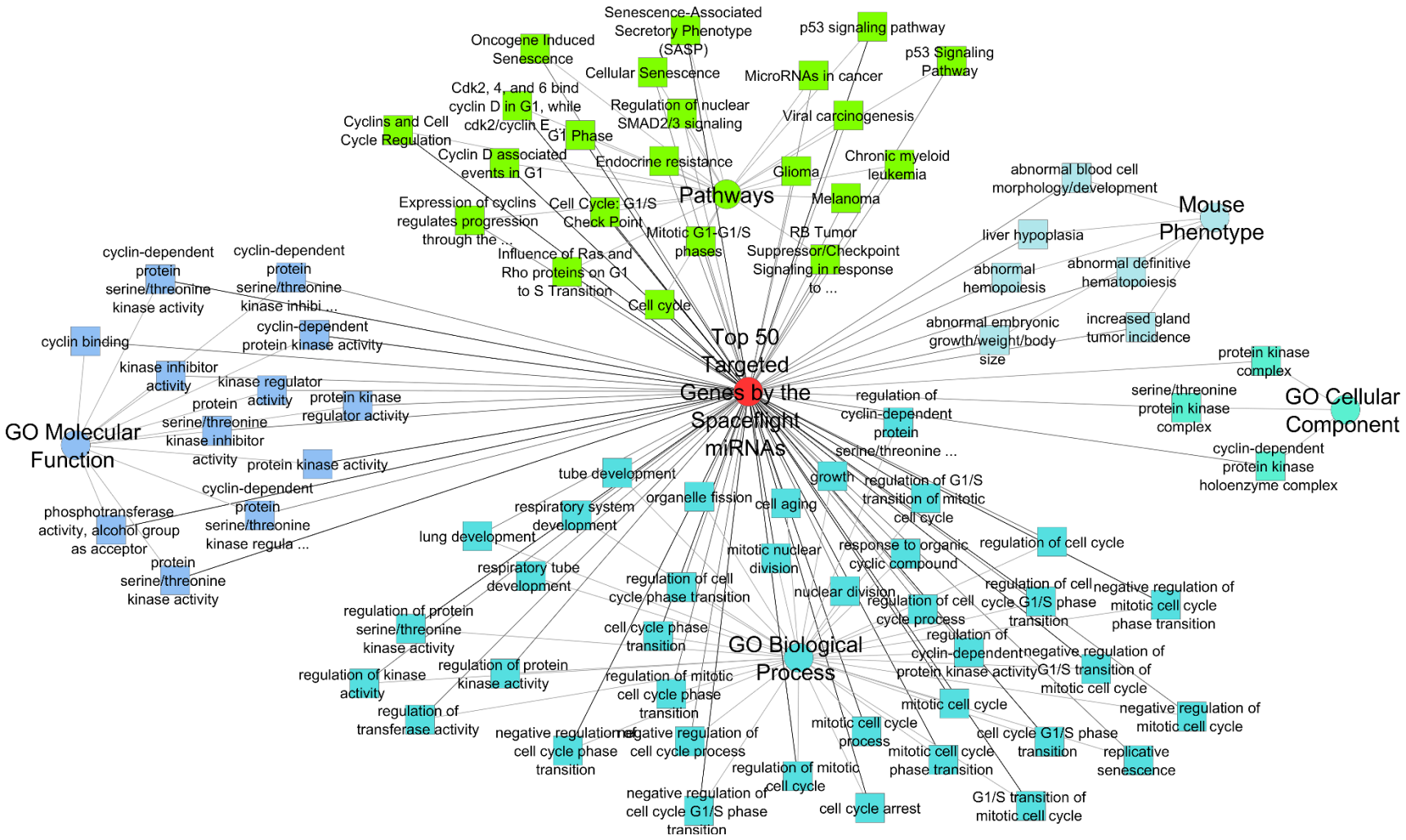

Figure S1. Predicted pathway and functional impact of the top 50 genes targeted by the spaceflight miRNAs, related to Figure 1. Pathways, functions, and phenotypes affected by the top 50 genes impacted by the spaceflight signature miRNA candidates were predicted by ToppCluster and displayed using Cytoscape.

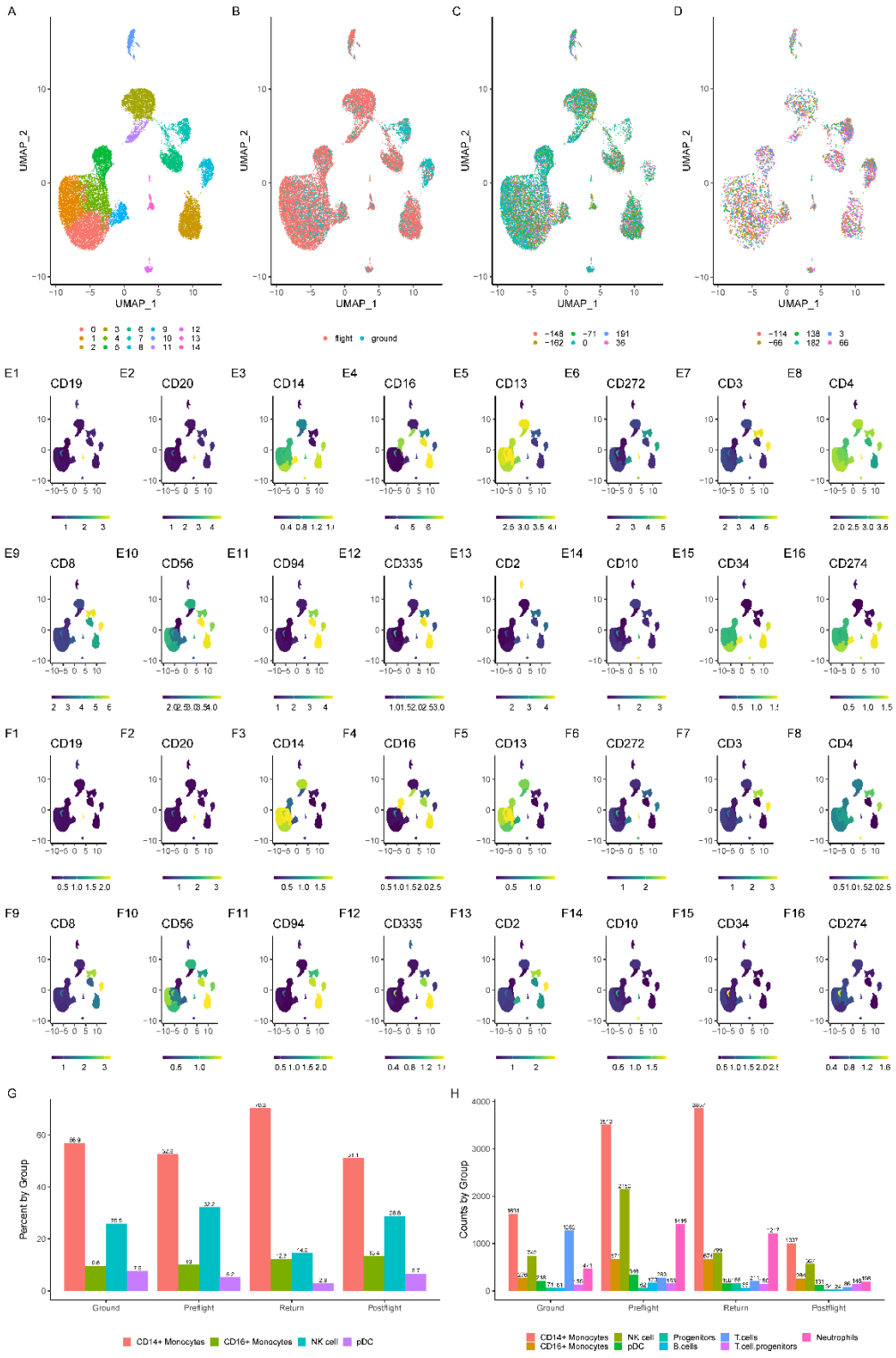

Figure S2. scRNA supporting data, related to Figures 5 and 6. A-D) UMAPs of Rhapsody data broken down by Seurat cluster (A), subject (B), TW sample grouped by sample date (C), and HR sample grouped by sample date (D). E) Average RNA expression of marker genes per cluster. F) Average protein expression of marker genes per cluster. G) Percent composition per group after removal of T and B cells. H) Number of cells per group.

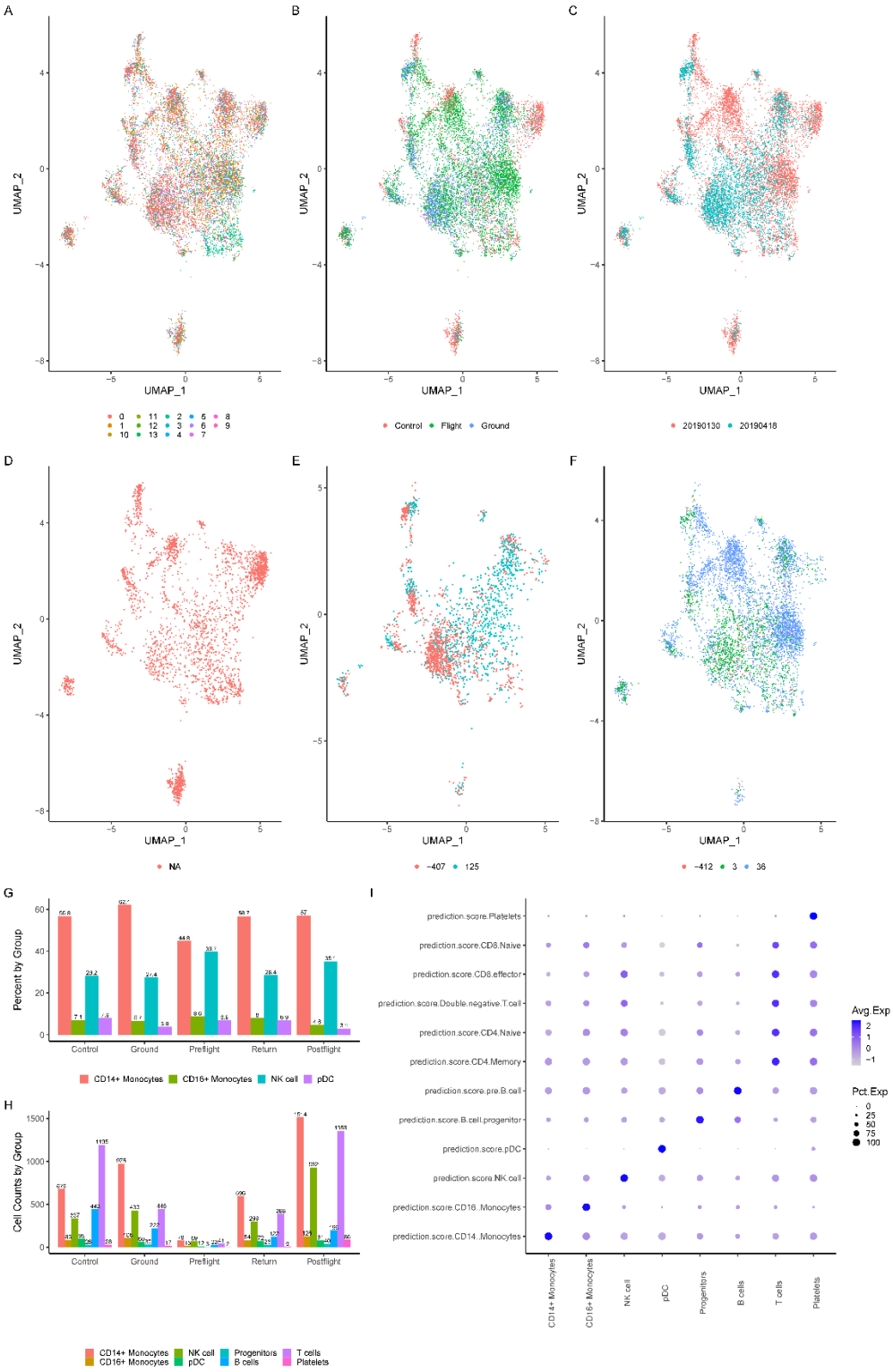

Figure S3. scATAC supporting data, related to Figure 6. A-F) UMAPs of scATAC data broken down by Seurat cluster (A), subject (B), experiment date (C), control PMBC cells (D), TW cells grouped by sample date (E), and HR cells grouped by sample date. G) Percent composition per group after removal of T and B cells. H) Number of cells per group. I) Dot plots showing the Seurat transfer anchor prediction scores grouped by cell identities.

Sample	Irradiation type	Loading	Age	Timepoint
Male Mice	Sham irradiation	Normal	16 weeks	11 days post-IR (n=10)
	Sham irradiation	HU	16 weeks	11 days post-IR (n=10)
	2 Gy Gamma (137Cs)	Normal	16 weeks	11 days post-IR (n=10)
	2 Gy Gamma (137Cs)	HU	16 weeks	11 days post-IR (n=10)
Male Mice	Sham irradiation	Normal	16 weeks	1 day post-IR (n=5) 11 days post-IR (n=9)
	Sham irradiation	HU	16 weeks	1 day post-IR (n=5) 11 days post-IR (n=9)
	1 Gy H (150 MeV/n)	Normal	16 weeks	11 days post-IR (n=9)
	1 Gy Fe-56 (600 MeV/n)	Normal	16 weeks	11 days post-IR (n=9)
	2 Gy Fe-56 (600 MeV/n)	Normal	16 weeks	11 days post-IR (n=6)
	1 Gy Mixed (0.5 Gy Fe-56 and 0.5 Gy 1H)	Normal	16 weeks	1 day post-IR (n=5) 11 days post-IR (n=9)
	1 Gy Mixed (0.5 Gy Fe-56 and 0.5 Gy 1H)	HU	16 weeks	1 day post-IR (n=5) 11 days post-IR (n=9)
Male Rats	None	20%	14 weeks	28 days of PWB (n=4)
	None	40%	14 weeks	28 days of PWB (n=4)
	None	70%	14 weeks	28 days of PWB (n=4)
	None	100%	14 weeks	28 days of PWB (n=8)
Female Mice	0.01128 Gy LEO exposure	μG	16 weeks	39 days post-launch (n=8)
	0.01128 Gy LEO exposure	μG	37 weeks	39 days post-launch (n=8)
	Ground	Normal	10 weeks	39 days post-launch (n=10)
	Ground	Normal	31 weeks	39 days post-launch (n=10)
Female Mice	0.5 Gy simGCRsim	HU	15 weeks	1 day post-IR (n=10)
	0.5 Gy simGCRsim	Normal	15 weeks	1 day post-IR (n=10)
	1 Gy simSPE	HU	15 weeks	1 day post-IR (n=10)
	1 Gy simSPE	Normal	15 weeks	1 day post-IR (n=10)
	5 Gy Gamma (137Cs)	HU	15 weeks	1 day post-IR (n=10)
	5 Gy Gamma (137Cs)	Normal	15 weeks	1 day post-IR (n=10)
	Sham	HU	15 weeks	1 day post-IR (n=10)
	Sham	Normal	15 weeks	1 day post-IR (n=10)
Male and Female Human PBMCs	Sham	Normal	20 - 64 years	4 hours post-IR (n=12)
	0.3 Gy Fe-56 600MeV/n	Normal	20 - 64 years	4 hours post-IR (n=12)

NASA Twin Study (Humans)	0.1463 Gy LEO exposure (over 340 days)	μG	50 years old	Before, during, and after flight (n=1)
	Ground	Normal	50 years old	During flight (n=1)
3D vascular model	Sham	Normal	-	48 hours post-IR (n=4)
	0.5 Gy SimGCRSim	Normal	-	48 hours post-IR (n=4)
	0.5 Gy SimGCRSim + Antagomirs	Normal	-	48 hours post-IR (n=4)
	0.5 Gy SimGCRSim + Scrambled Antagomirs	Normal	-	48 hours post-IR (n=4)

Table S1. Sample summary, related to Figures 1-7. miRNA content was quantified in serum or tissues from each of the experiments listed here. The experiments are listed in the order that they are presented in the paper. (IR = irradiation, HU = hindlimb unloaded, PWB = partial weight bearing, LEO = low-Earth orbit, μG = microgravity, SimGCRSim = 5-ion simplified galactic cosmic ray simulation, simSPE = solar particle event simulation, PBMCs = peripheral blood mononuclear cells).

miRNA target	Primer Annealing temperature	Catalog #
miR-125b-5p	58°C	Qiagen Cat# YP00205713
miR-145-5p	58°C	Qiagen Cat# YP00204483
miR-16-5p	58°C	Qiagen Cat# YP00205702
miR-21-5p	58°C	Qiagen Cat# YP00204230
miR-24-3p	58°C	Qiagen Cat# YP00204260
miR-26a-5p	58°C	Qiagen Cat# YP00206023
miR-34a-5p	50°C	Qiagen Cat# YP00204486
miR-17-5p	50.5°C	Qiagen Cat# YP02119304
miR-217-5p	50.5°C	Qiagen Cat# YP00205070
miR-223-3p	58°C	Qiagen Cat# YP00205986
let-7c-5p	58°C	Qiagen Cat# YP00204767
miR-92a-3p	58°C	Qiagen Cat# YP00204258
let-7a-5p	58°C	Qiagen Cat# YP00205727
miR-146a-5p	58°C	Qiagen Cat# YP00204688
miR-25-5p	51.8°C	Qiagen Cat# YP02118413

Table S2. Annealing temperatures for miRNA primers, related to methods and Figures 1-4, 7. Temperatures used for droplet digital PCR to quantify each miRNA target.

Age	Sex	Height (ft in)	Weight (lb)	BMI	Demographic (self-reported)
20	F	5ft 7in	200	31.3	Caucasian
25	F	5ft 2in	165	30.2	Caucasian
34	F	5ft 4in	235	40.3	Caucasian
39	M	5ft 9in	195	28.8	Caucasian
46	F	5ft 2in	153	28	Caucasian
48	F	5ft 7in	235	36.8	Caucasian
49	M	5ft 7in	180	28.2	Caucasian
51	M	5ft 9in	240	35.4	Caucasian
54	F	5ft 2in	180	32.9	Caucasian
63	F	5ft 2in	165	30.2	Caucasian
63	F	5 ft 3in	128	22.7	Caucasian
64	M	5ft 9 in	130	19.2	Caucasian

Table S3. Specific information on the donors for the PBMC samples utilized in Figure 4.