Electronic Supplementary Material (ESI) for Lab on a Chip.

WormSpace *μ-TAS* enabling automated on-chip multi-strain culturing and multi-function imaging of *Caenorhabditis elegans* at single-worm level on the China Space Station

Qianqian Yang[1#], Runtao Zhong[1#*], Wenbo Chang[1], Kexin Chen[1], Mengyu Wang[1], Shuqi Yuan[1], Zheng Liang[1], Wei Wang[1], Chao Wang[2], Guanghui Tong[3], Tao Zhang[3], Yeqing Sun[1*]

[1]Institute of Environmental System Biology, Dalian Maritime University, 116026 Dalian, China

[2]National Space Science Center, Chinese Academy of Sciences, 100190 Beijing, China

[3]Shanghai Institute of Technical Physics, Chinese Academy of Sciences, 200083 Shanghai, China

[#]Co-first author: Qianqian Yang, Runtao Zhong

*Correspondence to: Runtao Zhong, rtzhong@dlmu.edu.cn, Yeqing Sun, yqsun@dlmu.edu.cn

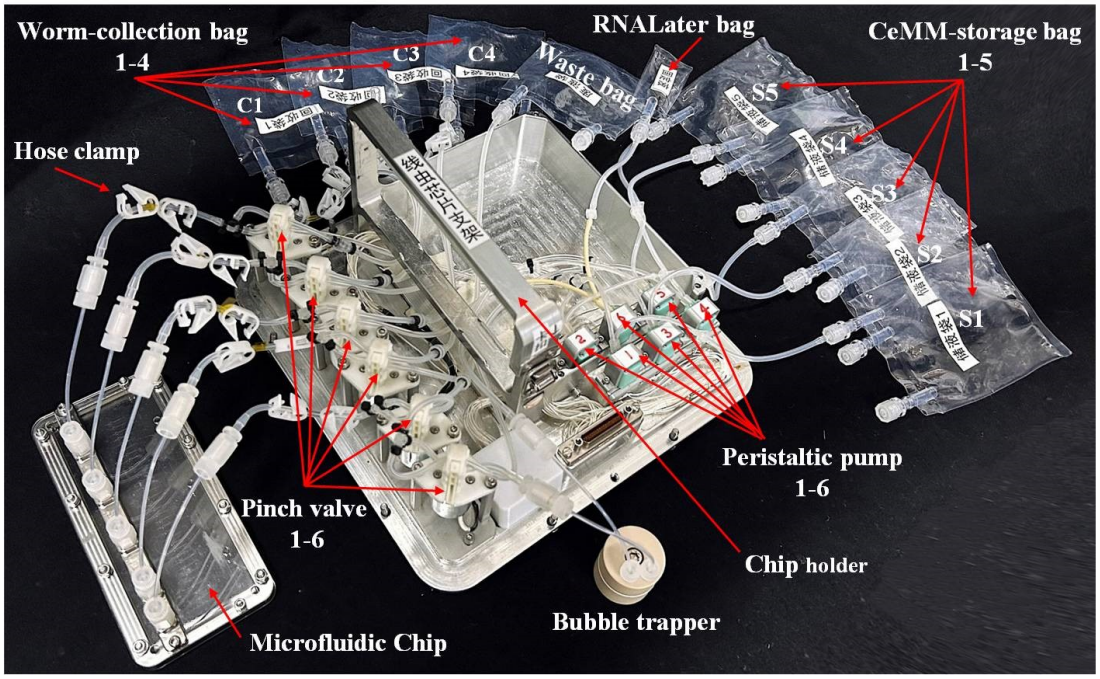

Fig. S1 Photograph of the components of the WormChip cartridge after connection of fluid lines.

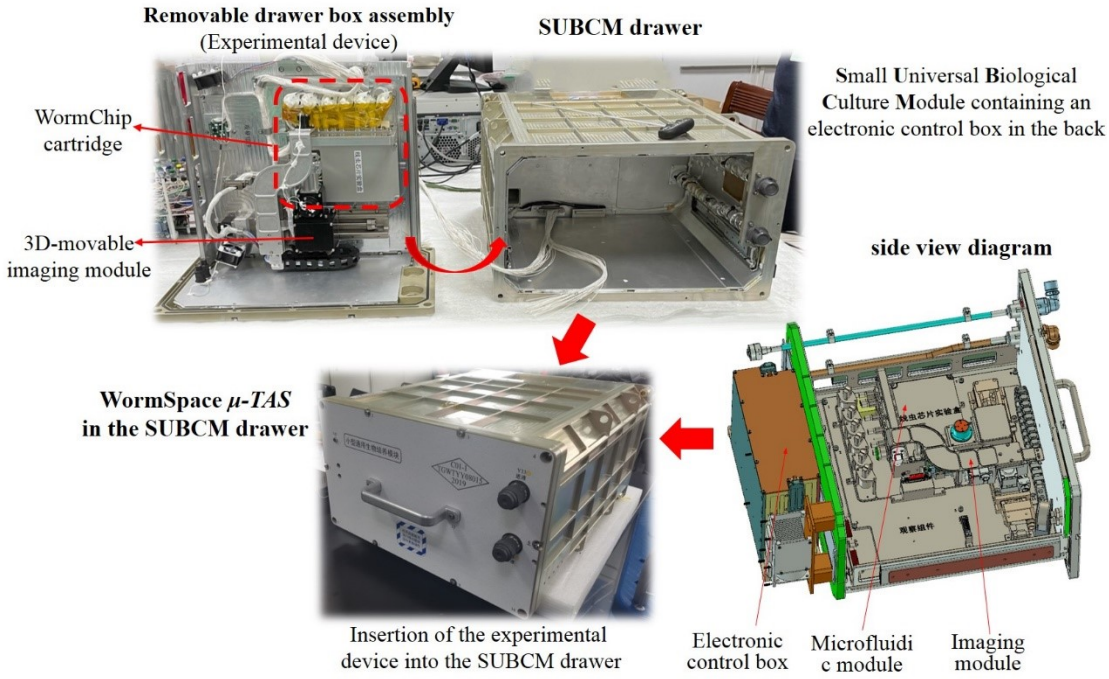

Fig. S2 Photographs and side view diagram showing the composition and structure of the WormSpace *μ-TAS*.

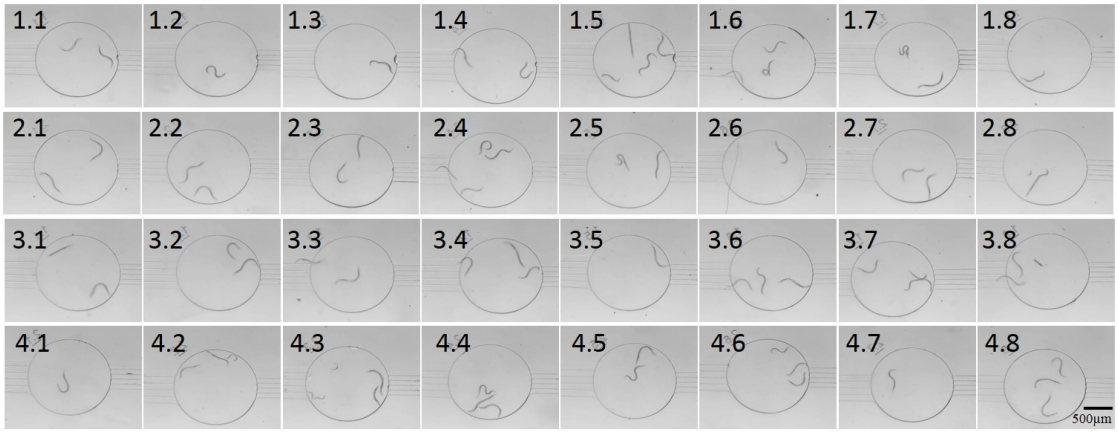

Fig. S3 Representative images of the culture chambers of the chip unit 1-4 with *C. elegans* strain N2 (Chamber 1.1-1.8), LS292 (Chamber 2.1-2.8), AM141 (Chamber 3.1-3.8) and TJ356 (Chamber 4.1-4.8) after sample loading during ground-based testing.

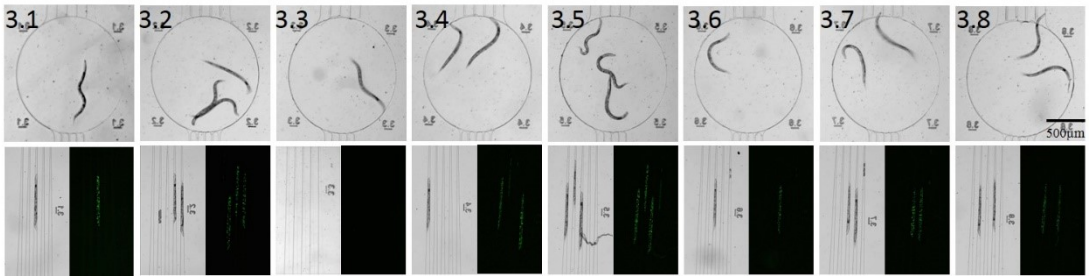

Fig. S4 Representative images of the chip unit-3 with *C. elegans* strain AM141 before (the upper bright-field images of the culture chambers) and after (the lower bright-field and fluorescent images of the worm-clamp arrays) worm immobilization during ground-based testing.

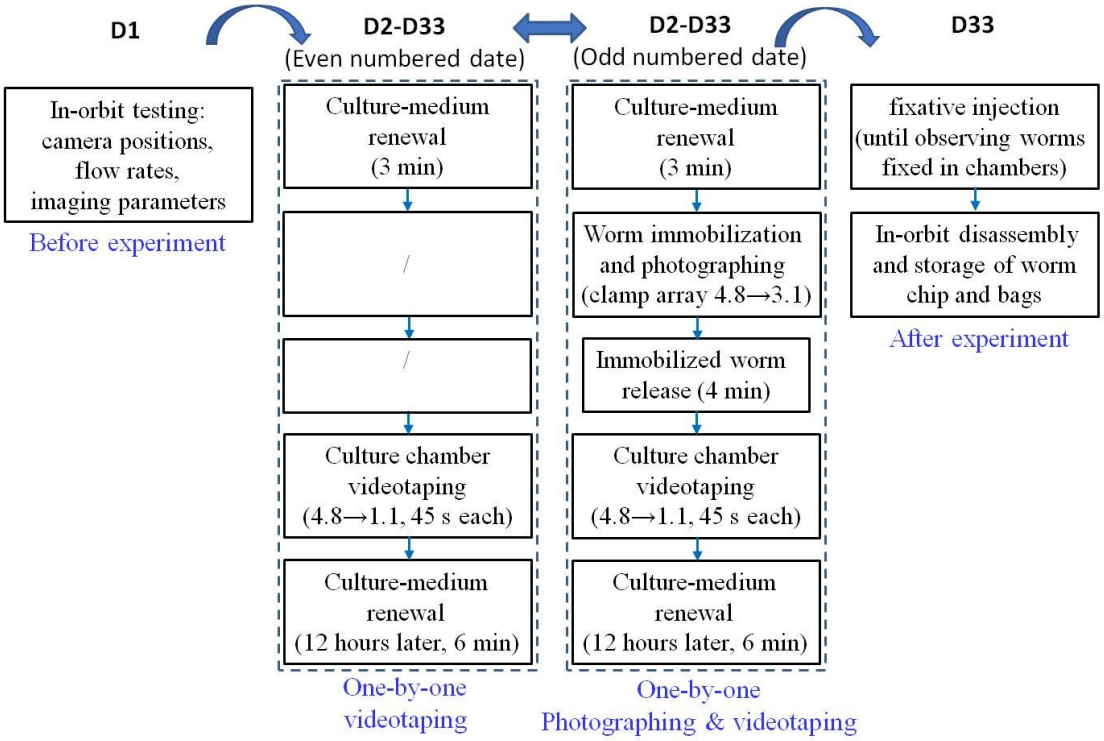

Fig. S5 Schematic diagram of the experimental protocols of automated *C. elegans* culturing and phenotypic monitoring on the CSS.

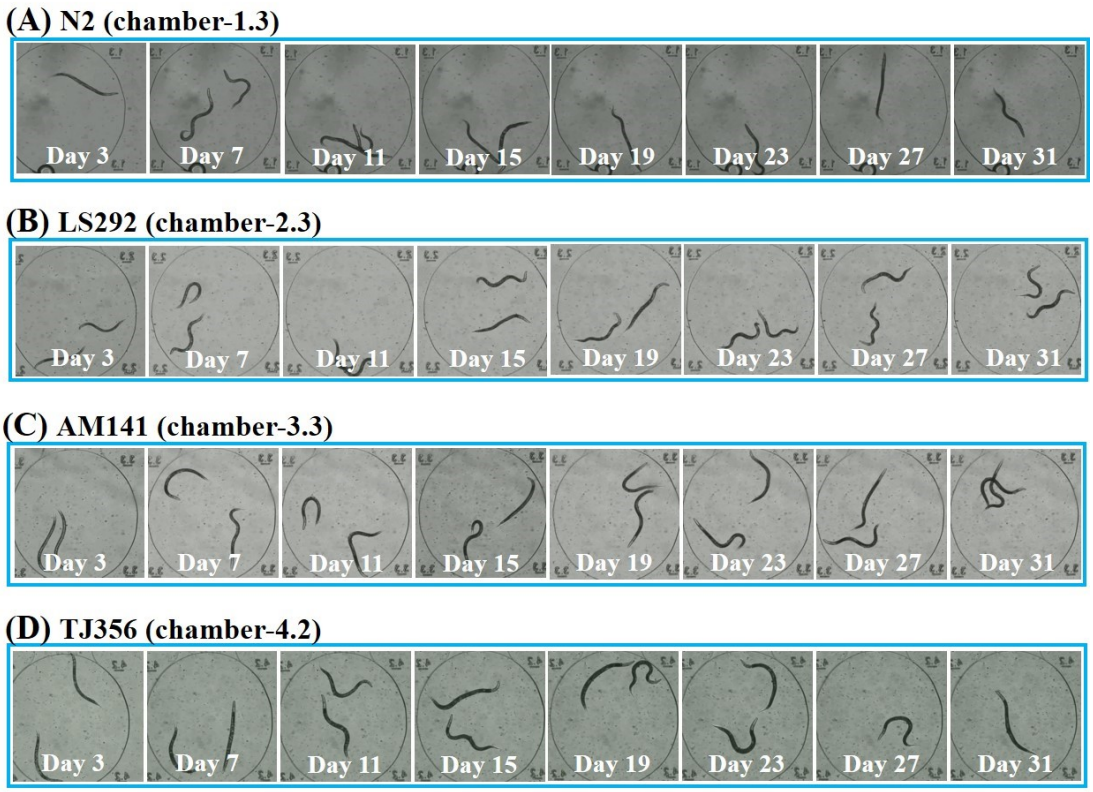

Fig. S6 Representative images of the *C. elegans* strain (A) N2, (B) LS292, (C) AM141 and (D) TJ356 within the culture chamber along the days of experiment on the CSS. The in-orbit testing before experiment was performed on Day 1, the culture chamber videotaping was performed on Day 2, and both photographing and videotaping was performed on Day 3.

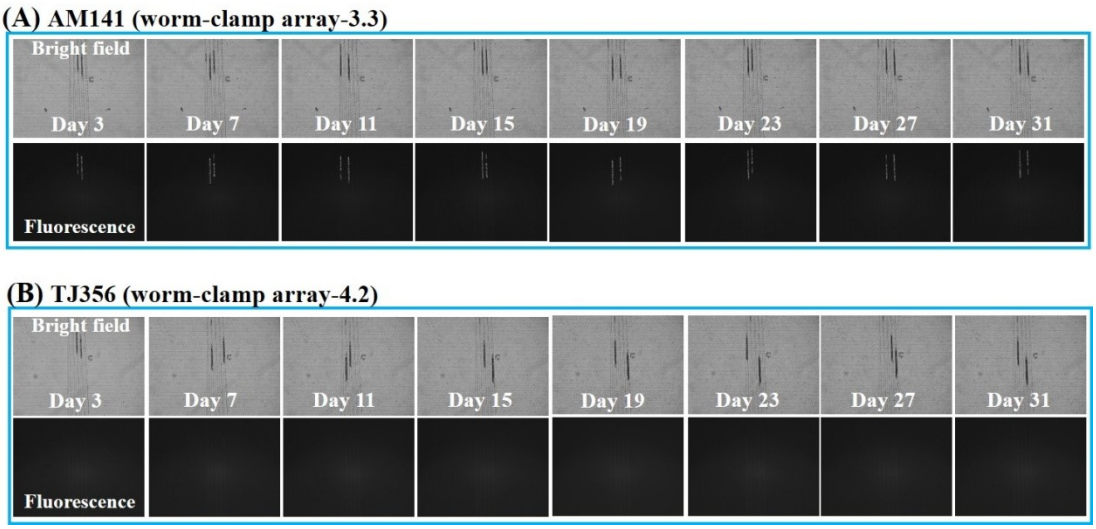

Fig. S7 Representative bright-field and fluorescent images of the *C. elegans* strain (A) AM141 and (B) TJ356 immobilized in the worm-clamp array along the days of experiment on the CSS. The in-orbit testing before experiment was performed on Day 1, the culture chamber videotaping was performed on Day 2, and both photographing and videotaping was performed on Day 3.

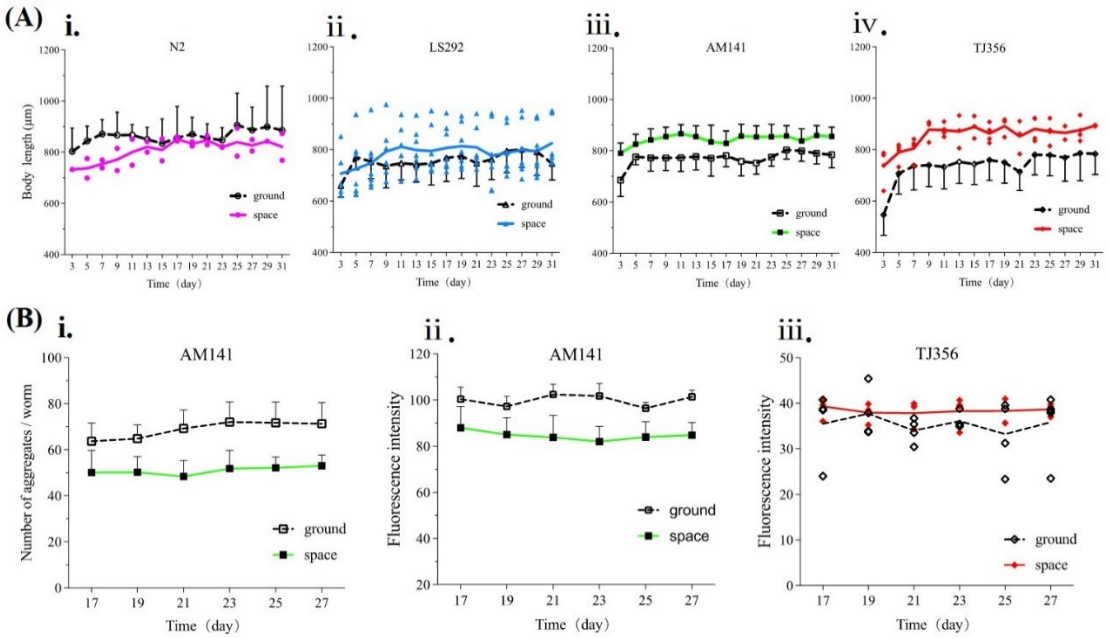

Fig. S8 Comparison of the results of on-chip culture growth and fluorescence obtained on the CSS to that obtained on earth during ground-based testing. (A) Changes of body length obtained from the videos of the 4 strains of *C. elegans* grown in CeMM within the 32 chambers of the WormChip-4.8.1 from Day 1 to Day 31. (i) N2 (n=1-2 for space and n≥12 for ground), (ii) LS292 (n≥5 for space and n≥13 for ground), (iii) AM141 (n≥12 for space and n≥10 for ground) and (iv) TJ356 (n=2-4 for space and n≥11 for ground). Error bars represent standard deviation. (B) Changes of fluorescence obtained from the fluorescent images of AM141 and TJ356 worms immobilized in the 8 worm-clamp arrays of chip unit-3 and unit-4, respectively, from Day 17 to Day 27. Changes of the number (i) and intensity (ii) of the Q40::YFP aggregates expressed in AM141 (n≥12 for space and n≥7 for ground), and (iii) intensity of the DAF-16::GFP aggregates expressed in TJ356 (n=2-4 for space and n≥4 for ground). Error bars represent standard deviation.

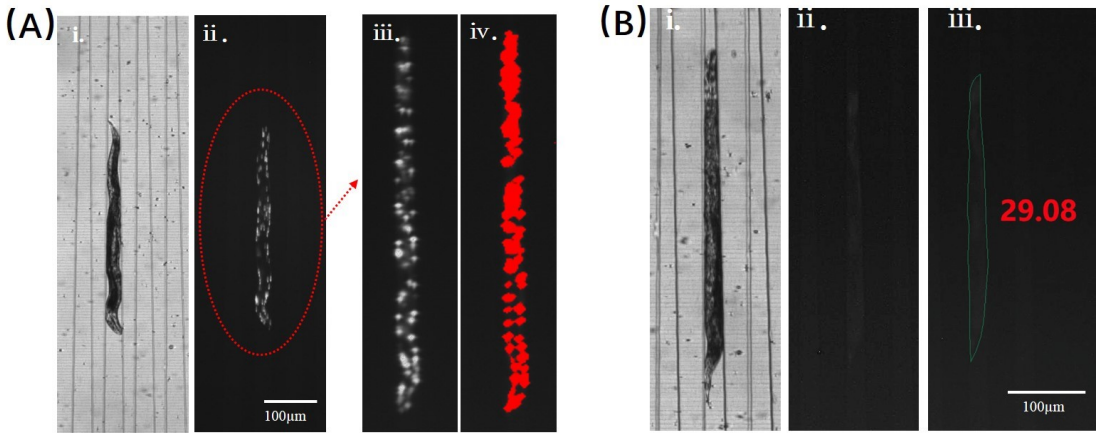

Fig. S9 Determination of the number of Q40::YFP aggregates (Aiii) and the average fluorescence intensity of fluorescent strain of AM141 (Aiv) and TJ356 (Biii) by using the ImageJ software and the fluorescence images obtained on orbit. The fluorescent region of each nematode worm in the images was selected either by software automatically (AM141, red area in Aiv), or by hand (TJ356) according to the bright-flied image, and then the average fluorescence density was calculated by the software.

Tab. S1 Comparison of bacterial diet and chemically defined medium (CeMM).

Culturing medium / **Features**	**Bacterial diet** (*E.coli* OP50)	**Chemically defined medium** (CeMM)
Liquid medium	√	√
Automated culturing and experimentation	×	√
Life span of cultured *C. elegans*≮30 days	×	√
Microfluidic chip-based spaceflight (removing clogging of microchannels)	×	√
Axenic cultivation	×	√
Removing theoretical health concerns to astronauts	×	√
Removing concerns of altered metabolism in flight	×	√
Reducing the oxygen exchange requirement	×	√

Tab. S2 Three fluid-control modes of the WormChip cartridge.

No.	Fluid-control mode	Purpose	Status of pinch valves (PVs)	Status of peristaltic pumps (PPs)	Direction of flow
1	Culture-medium renewal	Culture-medium renewal/ progeny collection/ trapped worm release	PV 1-5 switched on	PP-5 in operation (flow rate: ~ 60 µL/min)	CeMM-storage bag 5 → outlet → channels → inlet 1-4→ worm-collection bag 1-4
2	Worm immobilization	Trapping worms for fluorescence imaging	PV 1-6 switched off	PP 1-4 in operation (flow rates: ~ 20/20/20/30 µL/min for unit-4; ~20/20/60/30 µL/min for unit-3)	CeMM-storage bag 1-4 → inlet 1-4 → channels → outlet → waste bag
3	fixative injection	Injecting fixative into the culture chambers for *C. elegans* worms fixation	PV 1-6 switched on	PP-6 in operation (flow rate: ~ 60 µL/min)	RNALater bag → outlet → inlet 1-4→ tubings

Tab. S3 Ratio of chambers with worms and with single worm after the *C. elegans* loading process.

Number of loaded worms / **Chip no.**	**Unit-1# (Chamber 1.1-1.8)**	**Unit-2# (Chamber 2.1-2.8)**	**Unit-3# (Chamber 3.1-3.8)**	**Unit-4# (Chamber 4.1-4.8)**	**Ratio of chambers with worms (%)**	**Ratio of chambers with single worm (%)**
1#	21124311 **(15)**	22242121 **(16)**	12231332 **(17)**	13332313 **(19)**	100.00	31.25
2#	11344232 **(20)**	22412233 **(19)**	12420231 **(15)**	23222331 **(18)**	96.88	18.75
3#	42224144 **(23)**	23333114 **(20)**	21321023 **(14)**	14101121 **(11)**	93.75	31.25
Ave.	19.33	18.33	15.33	16	96.88	27.08

Tab. S4 Ratio of worms retained within the chambers on different days

Number of worms in chambers / Chip no.	Day 0	Day 1		Day 5		Day 10		Day 15	
	Number of worms	Number of worms	Retention efficiency (%)	Number of worms	Retention efficiency (%)	Number of worms	Retention efficiency (%)	Number of worms	Retention efficiency (%)
1#	99	99	100	78	78.8	78	78.8	78	78.8
2#	76	76	100	63	82.9	63	82.9	63	82.9
3#	84	84	100	81	96.4	81	96.4	81	96.4
Ave.	/	/	100	/	86.0	/	86.0	/	86.0

Tab. S5 Ratio of immobilized worms during the process of bright-field and fluorescence imaging.

Test no.	**Number of worms in chamber (3.1-3.8)**	**Bright-field imaging of worm-clamp array (3.1-3.8)**		**Fluorescence imaging of worm-clamp array (3.1-3.8)**	
		Number of worms	**Ratio of immobilized worms (%)**	**Number of worms**	**Ratio of immobilized worms (%)**
1#	13123122 **(15)**	02112112 **(10)**	66.67	02123122 **(13)**	86.67
2#	13123122 **(15)**	12013122 **(12)**	80	13023122 **(14)**	93.33
3#	13123112 **(14)**	12023112 **(12)**	85.71	13023112 **(13)**	92.86
Ave.	/	/	77.27	/	90.91

Tab. S6 Samples of the four strains of *C. elegans* for on-orbit culturing and phenotypic monitoring.

No. of chip unit	**1#**	**2#**	**3#**	**4#**
***C. elegans* strain**	N2	LS292	AM141	TJ356
Genotype	*Wild type*	*dys-1(cx18) I*	*rmIs133 [unc-54p::Q40::YFP]*	*zIs356 [daf-16p::daf-16a/b::GFP + rol-6(su1006)]*
Biological effects under space environment	-	Insensitive to microgravity	Sensitive to microgravity, muscular dystrophy	Sensitive to radiation
Fluorescence	-	-	Q40::YFP	DAF-16::GFP
Duration from synchronization to sampling	~ 5 d	~ 6 d	~ 6 d	~ 7 d
Samples for loading	Body width: 23-27 μm (Percentage of worms wider than 27 μm≯20%)			