#### **Supplementary Material**

Altered localization of ZmPIN1a proteins in plasma membranes responsible for enhanced-polar auxin transport in etiolated maize seedlings under microgravity conditions in space

Mariko Oka<sup>A,I</sup>, Motoshi Kamada<sup>B</sup>, Riko Inoue<sup>A</sup>, Kensuke Miyamoto<sup>C</sup>, Eiji Uheda<sup>D</sup>, Chiaki Yamazaki<sup>E</sup>, Toru Shimazu<sup>F</sup>, Hiromi Sano<sup>G</sup>, Haruo Kasahara<sup>H</sup>, Tomomi Suzuki<sup>H</sup>, Akira Higashibata<sup>H</sup> and Junichi Ueda<sup>D,I</sup>

<sup>A</sup>Faculty of Agriculture, Tottori University, 4-101 Koyamacho-minami, Tottori 680-8553, Japan.

<sup>B</sup>Future Development Division, Advanced Engineering Services Co., Ltd, 1-6-1 Takezono, Tsukuba, Ibaraki 305-0032, Japan.

<sup>c</sup>Faculty of Liberal Arts and Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan.

<sup>D</sup>Graduate School of Science, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan.

<sup>E</sup>JEM Mission Operations and Integration Center, Human Spaceflight Technology Directorate, Japan Aerospace Exploration Agency, 2-1-1 Sengen, Tsukuba, Ibaraki 305-8505, Japan.

<sup>F</sup>Technology and Research Promotion Department, Japan Space Forum, Shin-Otemachi Bldg., 2-2-1 Otemachi, Chiyoda-ku, Tokyo 100-0004, Japan.

<sup>G</sup>Utilization Engineering Department, Japan Manned Space Systems Corporation, Space Station Test Building, Tsukuba Space Center, 2-1-1 Sengen, Tsukuba, Ibaraki 305-8505, Japan.

<sup>H</sup>Kibo Utilization Center, Human Spaceflight Technology Directorate, Japan Aerospace Exploration Agency, 2-1-1 Sengen, Tsukuba, Ibaraki 305-8505, Japan.

<sup>I</sup>Corresponding authors. Email: m.oka@tottori-u.ac.jp; ueda@b.s.osakafu-u.ac.jp



**Fig. S1.** Effect of  $\mu g$  conditions in space on the accumulation of ZmPIN1a proteins in etiolated maize seedlings. The amount of ZmPIN1a proteins in the coleoptile tip, subapical region of the coleoptiles, the mesocotyls, the primary leaf, the root tip, and the root were determined using western blotting analysis with designing specific polyclonal antibody of ZmPIN1a. a: Eight  $\mu g$  of total proteins were loaded into each lane and separated through SDS-PAGE. After SDS-PAGE and blotting of the gel onto PVDF membrane, the signal of ZmPIN1a proteins was detected with affinitypurified anti-ZmPIN1a antibodies. b: Relative intensity of ZmPIN1a proteins. Data are shown as mean  $\pm$  s.e. (n = 3). Asterisks denote the significance of 2-way ANOVA: \*\*P < 0.01, \*P < 0.05, ns: not significant. Abbreviations indicate each factor: G: gravity conditions, O: organ differences. Different letters denote significant differences among independent samples (P < 0.05, Tukey test).

### Table S1. Two-way ANOVA of the effects of gravity conditions and organ differences on the accumulations of *ZmPIN1a* mRNA for the data in Fig. 1a

	Df	Sum Sq	Mean Sq	F-value	<i>P</i> -value
G	1	0.1938	0.1938	0.4317	0.5236
О	2	2.0314	1.0157	2.2622	0.1467
G×O	2	3.8624	1.9312	4.3012	0.0391*

## Table S2. Two-way ANOVA of the effects of gravity conditions and organ differences on the accumulations of *ZmAUX* mRNA for the data in Fig. 1b

	Df	Sum Sq	Mean Sq	F-value	P-value
G	1	0.0166	0.0166	0.2705	0.6125
0	2	0.5992	0.2996	4.8952	0.0279 *
G×O	2	0.4296	0.2148	3.5099	0.0631 '

# Table S3. Two-way ANOVA of the effects of gravity conditions and organ differences on the amounts of ZmPIN1a proteins for the data in Fig. 2

	Df	Sum Sq	Mean Sq	F-value	P-value
G	1	2.0269	2.0269	13.8773	0.0029**
0	2	0.0754	0.0377	0.2581	0.7767
G×O	2	0.0083	0.0041	0.0284	0.9721

## Table S4. Two-way ANOVA of the effects of gravity conditions and organ differences on the amounts of ZmPIN1a proteins for the data in Supplementary Fig. 1

	Df	Sum Sq	Mean Sq	F-value	P-value
G	1	2.1275	2.1275	27.1155	2.461e-05***
0	5	5.6041	1.1208	14.2850	1.563e-06***
G×O	5	0.3822	0.0764	0.9741	0.4534

## Table S5. Two-way ANOVA of the effects of gravity conditions and organ differences on the accumulations of *ZmPIN1c* mRNA for the data in Supplementary Fig. 2

	Df	Sum Sq	Mean Sq	F-value	<i>P</i> -value
G	1	0.9660	0.9660	3.9466	0.0703 '
0	2	8.3378	4.1689	17.0318	0.0003 ***
G×O	2	1.7539	0.8770	3.5827	0.0603 '