High-dose Ascorbate Exhibits Anti-proliferative and Anti-invasive Effects Dependent on 1 PTEN/AKT/mTOR Pathway in Endometrial Cancer in vitro and in vivo 2 Xiaochang Shen<sup>1,2\*</sup>, Jiandong Wang<sup>1\*</sup>, Weimin Kong<sup>1</sup>, Catherine John<sup>2</sup>, Boer Deng<sup>1,2</sup>, Shuning 3 Chen<sup>1,2</sup>, Haomeng Zhang<sup>1,2</sup>, Jennifer Haag<sup>2</sup>, Nikita Sinha<sup>2</sup>, Wenchuan Sun<sup>2</sup>, Angeles Alvarez 4 Secord<sup>3</sup>, Chunxiao Zhou<sup>2,4\*\*</sup>, and Victoria L Bae-Jump<sup>2,4\*\*</sup> 5 1. Department of Gynecological Oncology, Beijing Obstetrics and Gynecology Hospital, 6 7 Capital Medical University, Beijing Maternal and Child Health Care Hospital, Beijing 100006, PR China. 8 2. Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, 9 University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. 10 3. Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Duke 11 Cancer Institute, Duke University, Durham, NC 27710, USA. 12 4. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, 13 Chapel Hill, NC 27599, USA. 14 \* Both authors contributed equally to this work 15 \*\*Corresponding author 16 Victoria L. Bae-Jump MD PhD Chunxiao Zhou MD PhD 17 18 Professor Associate Professor Division of Gynecologic Oncology Division of Gynecologic Oncology 19 University of North Carolina at Chapel Hill University of North Carolina at Chapel Hill 20 CB# 7572, Physicians Office Building Rm# B105 CB#7295, Lineberger Cancer Center, RM#31-340 21 170 Manning Drive 450 West Drive 22 Chapel Hill, NC 27599. USA Chapel Hill, NC 27599. USA 23 Tel: 919-843-4899 Tel: 919-966-3270 24 Fax: 919-966-2646, Fax: 919-966-2646 25 Email: victoria baejump@med.unc.edu Email: czhou@med.unc.edu 26 27 28 29 30 31 32 33

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**Supplementary Table 1** Statistical comparison of IC50 of six cell lines

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Names	IC50 (mM) $\pm$ SD	KLE	Hec-1B	Ishikawa	RL-952	AN3CA	EC-023
KLE	$1.78\pm0.26$	-	ns	ns	**	*	*
Hec-1B	$1.94\pm0.21$		-	ns	**	*	*
Ishikawa	$2.95\pm0.88$			-	ns	**	**
RL-952	$3.66 \pm 0.57$				-	**	**
AN3CA	$0.48\pm0.07$					-	ns
EC-023	$0.33\pm0.25$						-

37 \**p*<0.05, \*\**p*<0.01, ns: not significant.



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## Supplementary Figure 1. The safety profiles of ascorbate in *LKB1<sup>f1/f1</sup>p53<sup>f1/f1</sup>*-transgenic mouse model

The body weight of mice was measured once a week. Results indicate that ascorbate treatment
(oral and IP) or combined with paclitaxel for 4 weeks had no significant impact on body weight

43 in both HFD and LFD groups (A, B). The administration of NAC alone or combined with

44 ascorbate (IP) for 4 weeks did not affect the body weight in the LFD group (C). H&E staining

of tissues from the heart, lung, liver, spleen, and kidney demonstrated no morphological
changes in major organs after treatment of ascorbate, paclitaxel, and the combination for 4
weeks in the HFD and LFD groups (D). Asc: Ascorbate, HFD: High-fat diet, LFD: Low-fat
diet. C (Oral) = Vehicle control for oral administration group, C (IP) = Vehicle control for IP
injection group.



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51 Supplementary Figure 2. The quantitative analysis for Western blotting (Figure 1 to 52 Figure 5)

53 The quantitative analysis of protein expression for western blotting was conducted using image

54 Lab software and normalized to appropriate internal controls. The quantitative analysis for

Figure 1E (A, B). The quantitative analysis for Figure 2B (C). The quantitative analysis for Figure 2C (D). The quantitative analysis for Figure 3D (E). The quantitative analysis for Figure 4E (F). The quantitative analysis for Figure 4L (G). The quantitative analysis for Figure 5A (H). The quantitative analysis for Figure 5B (I). The quantitative analysis for Figure 5H (J). Asc: Ascorbate, HFD: High-fat diet, LFD: Low-fat diet. C (IP) = Vehicle control for IP injection group. \*p<0.05, \*\*p<0.01 compared with C or vehicle control. \*p<0.05, \*\*p<0.01 compared with each group.



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Supplementary Figure 3. The quantitative analysis for Western blotting (Figure 6 to
Figure 7)

The quantitative analysis of protein expression for western blotting was conducted using image Lab software and normalized to appropriate internal controls. The quantitative analysis for Figure 6D (A). The quantitative analysis for Figure 6H (B). The quantitative analysis for Figure 7A (C). The quantitative analysis for Figure 7F (D). Asc: Ascorbate, \*p<0.05, \*\*p<0.01compared with C. #p<0.05, #p<0.01 compared with each group.