

Supplementary data for

## **Storage Stability of Plant Leaf-Derived Extracellular Vesicles According to Preservative and Storage Temperature**

Kimin Kim <sup>1</sup>, Jungjae Park <sup>2</sup>, Yehjoo Sohn <sup>1</sup>, Chan-Eui Oh <sup>1</sup>, Ji-Ho Park <sup>3</sup>, Jong-Min Yuk <sup>2</sup> and Ju-Hun Yeon <sup>1,\*</sup>

<sup>1</sup> Department of Integrative Biosciences, University of Brain Education,  
Cheonan 31228, Korea; kimini1127@naver.com (K.K.); spielian@naver.com (Y.S.);  
97rhdaud@naver.com (C.-E.O.)

<sup>2</sup> Department of Materials Science and Engineering, Korea Advanced Institute of Science and  
Technology, Daejeon 34141, Korea; jungjae10@kaist.ac.kr (J.P.);  
jongmin.yuk@kaist.ac.kr (J.-M.Y.)

<sup>3</sup> Department of Bio and Brain engineering, Korea Advanced Institute of Science and  
Technology, Daejeon 34141, Korea; jihopark@kaist.ac.kr

\* Correspondence: jhyeon@ube.ac.kr; Tel.: +82-41-529-2621; Fax: +82-41-529-2674

	Temperature		-20 °C			4 °C			25 °C			45 °C		
Condition	Date	N	Odor	Color	Phase Separation	Odor	Color	Phase Separation	Odor	Color	Phase Separation	Odor	Color	Phase Separation
LEVs	0 w	3	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change
	1 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	2 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	3 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	4 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	5 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C

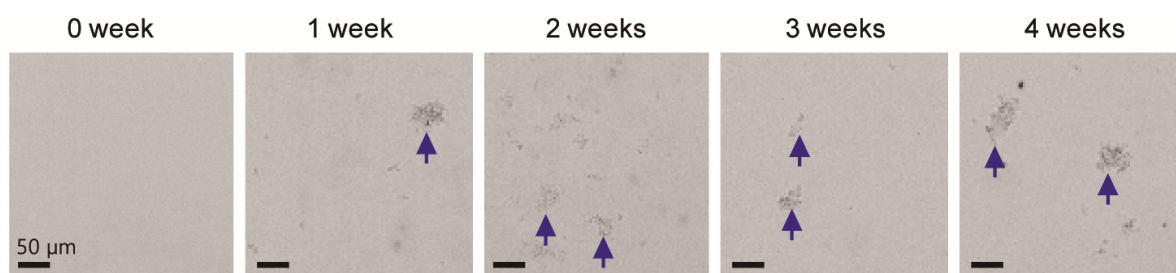
**Supplementary Table S1. Physical characteristics of LEVs.**

	Temperature		-20 °C			4 °C			25 °C			45 °C		
Condition	Date	N	Odor	Color	Phase Separation	Odor	Color	Phase Separation	Odor	Color	Phase Separation	Odor	Color	Phase Separation
LEVs -1,3BG	0 w	3	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change
	1 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	Soluble sediment
	2 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	Soluble sediment
	3 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	Soluble sediment
	4 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	Soluble sediment
	5 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	Soluble sediment

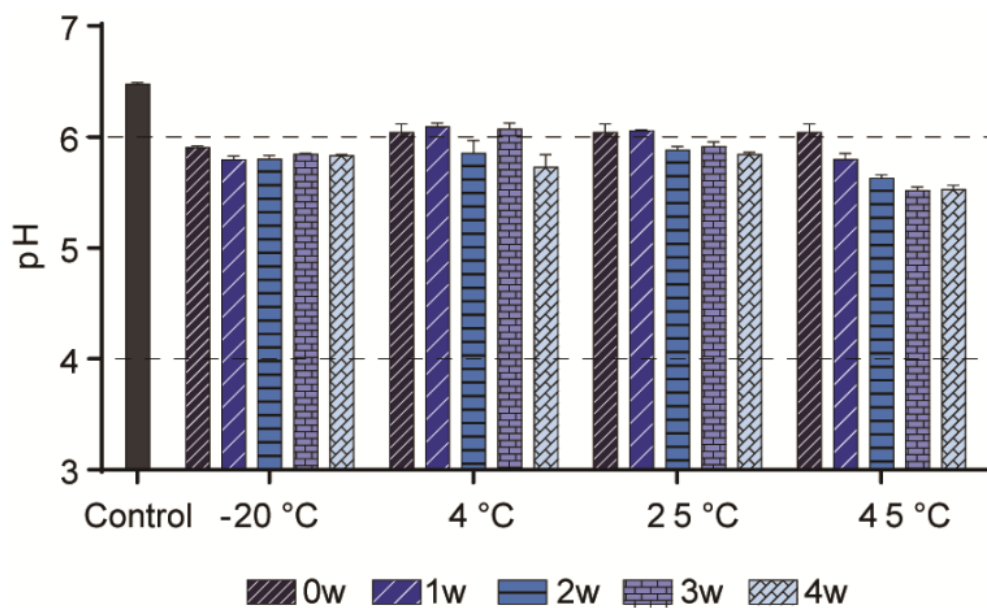
**Supplementary Table S2. Physical characteristics of mixture of LEVs and 1,3 BG.**

	Temperature		-20 °C			4 °C			25 °C			45 °C		
Condition	Date	N	Odor	Color	Phase Separation	Odor	Color	Phase Separation	Odor	Color	Phase Separation	Odor	Color	Phase Separation
LEVs -TMO	0 w	3	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change	Slightly characteristic odor	Light yellow	No change
	1 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	2 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	3 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	4 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C
	5 w	3	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C	N.C

**Supplementary Table S3. Physical characteristics of mixture of LEVs and TMO.**



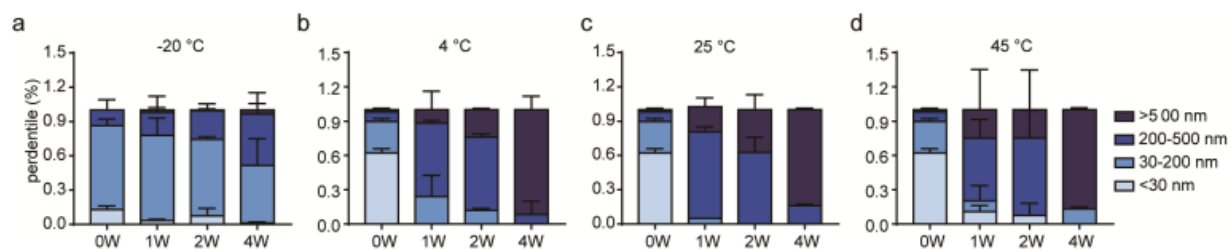
**Supplementary Figure S1. Optical microscopy images of mixture of LEVs and 1,3-BG using at 45°C during 4 weeks.** The mixture of LEVs and 1,3-BG showed soluble sediment at 45°C from 1 weeks to 4 weeks.



**Supplementary Figure S2. pH tests of LEVs-1,3BG over time.**

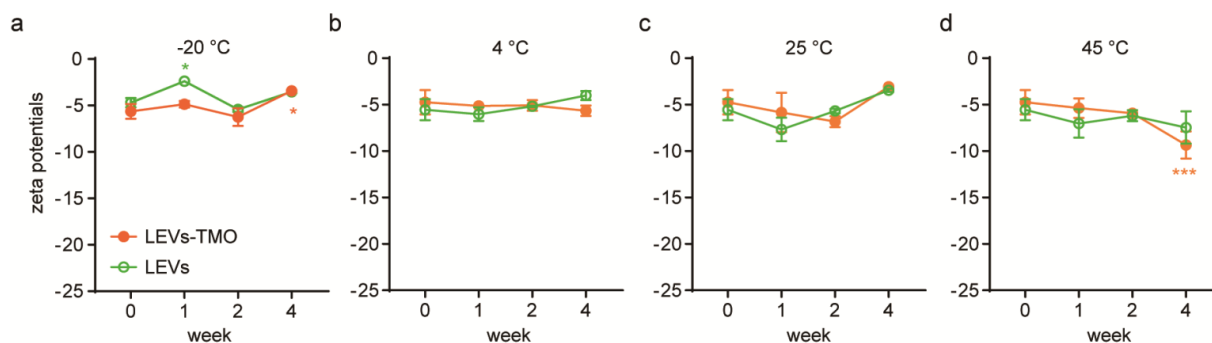
	LEVs	LEVs+1,3BG	LEVs+TMO
PDI	$0.238 \pm 0.105$	$0.270 \pm 0.045$	$0.270 \pm 0.060$

**Supplementary Figure S3. PDI values of LEVs, LEVs-1,3BG, and LEVs-TMO.**



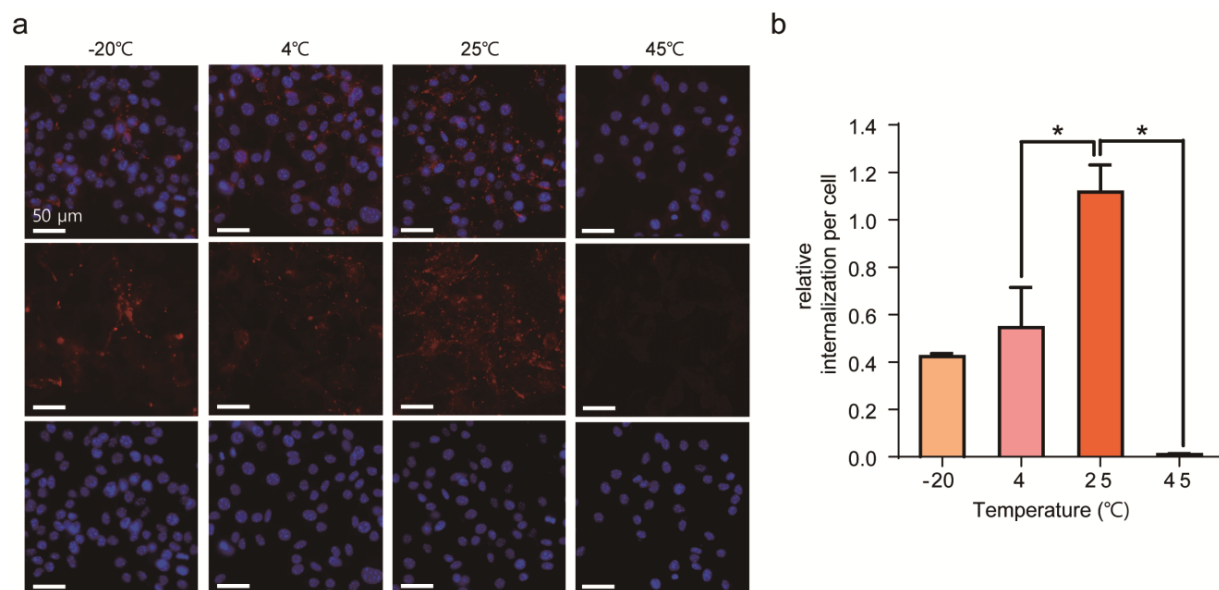
**Supplementary Figure S4. Size distribution of LEVs-1,3BG over time under different temperature.** The mixture of LEVs and 1,3-BG showed change of size distribution stored at -20, 4, 25, 45 °C.





**Supplementary Figure S5. Zeta potentials over time under different temperatures.**

Zeta potentials of LEVs and LEVs-TMO stored at (a) -20, (b) 4, (c) 25, and (d) 45 °C for 4 weeks. Data are presented as mean  $\pm$  standard error of mean (SEM). (\*\*\*)  $p < 0.001$ , (\*\*\*\*)  $p < 0.0001$ .



**Supplementary Figure S6. Cellular uptake for LEVs under different temperatures.** (a) Representative fluorescence microscopic images of cellular uptake for LEVs stored at -20, 4, 25, and 45 °C. (b) Summary data comparing intracellular fluorescence intensity per cell. Data are presented as mean  $\pm$  standard error of mean (SEM). (\* $p < 0.01$ ).