

Supplementary data: Characterization of nanovesicles derived from plant juices

Stefania Raimondo et al. 2015	<i>Citrus limon</i>	3,000 × g for 30 min, 10,000 × g for 1h. The supernatant was filtered at 0.8 µm and 0.45 µm, centrifuged at 16,500 × g for 3 h, at 120,000 × g for 90 min. The pellet was transferred to a 30% sucrose/D2O cushion. Vesicles contained in the cushion were washed for 90min at 120,000 × g	50–70 nm; 600 micrograms of vesicles from 240 ml of Citrus juice; density 1,15 to 1,19 g/ml	ND		580 proteins	contain small RNAs	_lung carcinoma cell line A549 _chronic myeloid leukemia cell line LAMA84 cells	cancer cell proliferation inhibition	50 µg of DIR-labeled EVs in 150 µl PBS for in vivo imaging; 50 µg/mouse, 3 days a week for 2 weeks administered intraperitoneally or in the intratumor
Songwen Ju et al. 2013	Grapes	Differential centrifugations and then centrifugation on a sucrose gradient	380.5 ± 37.47 nm	-69.6 mV to +2.52 mV	phosphatidic acid (53.2%) and phosphatidylethanolamine (26.1%)	28 proteins	96 miRNAs	incorporated through macropinocytosis in: _Intestine of Lgr5-EGFP-IRES- CreERT2-mice. _intestinal epithelial cell line CT26	induction of intestinal stem cells	1mg of PHK26-labeled EVs in 200 µl PBS for gavage; 1mg of DIR-labeled EVs in 200 µl PBS for in vivo imaging; Gavage with 2mg/mouse for 7 days for stem cells
Pedro Pérez-Bermúdez et al. 2017	Grapes	16,000g for 10min. Juice was concentrated with Centricon Plus-70 (Millipore) at 3500g for 40min, concentrated juice as filtered at 0.22microns. vesicles were pellet and wash at 120,000g for 1h				121 proteins				
Mingzhen Zhang et al. 2016	Ginger	Ginger juice centrifugated at 3,000g for 20min, 10,000g for 40min, 150,000g for 2h and the pellet was resuspended in PBS and sonicated. Then spun at 150,000g for 2h in a sucrose gradient (8%,30%,45%,60%). Band 1 at the 8/30% interface Band 2 at the 30/45% interface Band 3 at 45/60% interface Band1/2 resist to freeze/thaw	Band 1: 292.5nm; Band 2: 231.6nm; Band 3: 219.6nm	Band 1/ 2: -12mV Band 3: 0mV	phosphatidic acid (25-45%), digalactosyldiacylglycerol (25-40%) Band 2 contain the ginger active constituent 6-gingerol and 6-shogaol		125 miRNAs			0.3mg/mouse every day orally administered

Baomei Wang et al. 2014	Grapefruit	Differential centrifugations (2,000g for 20 min ,10,000g for 1 hour), EVs were pelleted at 150,000g for 1.5 hours, washed once with PBS, and then separated using sucrose gradients (8%,30%,45%,60%,). Band 1 at the 8/30% interface and band 2 at the 30/45% interface were separately. Only EVs from band 1 of the gradient were selected (8/30% interface)	210.8 ± 48.62 nm	-49.2 to -1.52 mV	phosphatidylethanolamine (45.52%) and phosphatidylcholine (28.53%). In particular, phosphatidylethanolamine (34:2) and phosphatidylcholine (34:2) are highly enriched	136 peptides	ND	Incorporated via both macropinocytosis and clathrin-dependent pathways in Raw264.7 macrophages	enhance anti-inflammatory capacity of intestinal macrophages	30 mg/kg of PHK26-labeled EVs or DiR-labeled EVs for gavage for imaging; or 10mg/kg of EVs daily for 7 days to test EV' anti-inflammatory effect in mice
Qilong Wang et al. 2015	Grapefruit	as in Baomei Wang et al. 2014	as in Baomei Wang et al. 2014	as in Baomei Wang et al. 2014	as in Baomei Wang et al. 2014	ND	ND	ND	Binding membranes derived from the PMA-activated EL4 T-cell line to grapefruit-derived EVs to obtain artificially coated EVs with inflammatory-related receptor enriched membranes	ND
yingyao Mu et al. 2014	Grape, Grapefruit, Ginger, Carrot	1000 × g for 10 min, 3000 × g for 20 min, and 10 000 × g for 40 min to remove large particles. The supernatant was 150 000 × g for 90 min, the pellet was resuspended in 1 mL PBS and transferred to a sucrose step gradient (8%/15%/30%/45%/60%) and centrifuged at 150 000 × g for 120 min. Only sucrose gradient purified band 2 is further considered	Grape (150 -1000nm); Grapefruit (50 -1000nm); Ginger (100-1000nm); Carrot (90-110nm and 800-1000nm). These sizes are smaller in acidic conditions	negative	ND	ND	grape EVs contain miRNAs enriched for the miR-169 family	intestinal stem cells of Lgr5-EGFP-IRES-CreERT2 mice or RAW 264.7 macrophages	All plant EVs induce nuclear translocation of macrophage Nrf2 and intestinal Wnt/TCF4 activation of mice orally administrated EVs	gavage twice a day for 3 days with 2 mg of EVs per mouse in 200 µl PBS; gavage with 1 mg per mouse in 200 µl PBS of PKH26 dye labeled EVs for Intestinal macrophages and LGR5 stem cells up-take imaging
Mariana Regente et al. 2009	Sunflower seeds ( <i>H. annuus</i> L., line 10347 Advanta Semillas)	Seed imbibition during 2 h in water then peeled to remove the pericarp; immersed in 50 mM Tris-HCl pH 7.5, 0.6% NaCl, 0.1% 2-mercaptoethanol and subjected to three vacuum pulses of 10 s, separated by 30 s intervals. Extracellular fluid were filtrated through a 0.5 µm membrane and subjected to centrifugations (10 000×g for 30 min, 40 000×g for 60 min and 100 000×g for 60 min). The 40 000×g and 100 000×g pellet fractions were suspended in 30 µl of 20 mM Tris-HCl pH 7.5.	50–200 nm in both 40 000×g and 100 000×g pellets	ND	phosphatidic acid (PA)and phosphatidylinositol phosphate	similar protein patterns in the 40 000×g and 100 000×g pellets. Presenec of agglutinin I; FSGTP1 (68% homology with human Rab11A and 81% with Arabidopsis thaliana Rab GTPase 11C0);	ND	ND	ND	ND

Xiaoying Zhuang et al. 2015	Ginger	Ginger juice differentially centrifuged (1,000g for 10 min, 3,000g for 20 min and 10,000g for 40 min). 10,000g pellet was referred to as microparticles. The supernatant was centrifuged at 150,000g for 90 min and transferred to a sucrose step gradient (8%/30%/45%/60%) and centrifuged at 150,000g for 120 min. 2 bands; band 1 (between 8%/30% layers) and the band 2 (between 30%/45% layers)	Band 1: 386.6 nm; Band 2: 294.1 nm. These sizes are larger in acidic conditions. For 200 g of ginger roots, Band 1= 3.79 ± 0.27 mg/g ; Band 2= 0.319±0.01 mg/g	Band 1: 24.6 mV; Band 2: 29.7 mV Changed from negative to a positive charge at pH=2;	phosphatidic acids (PA), digalactoyldiacylglycerol and monogalactosyl monoacylglycerol. shogaols much higher in Band 1	ND	ND	EVs from Ginger are internalized in primary hepatocytes through endocytosis	Ginger' EVs increase nuclear translocation of Nrf2 in targeted hepatocytes. This effect is associated with the concentration of EV' shogaol. Mice gavaged with Ginger' EVs had decreased liver triglyceride levels, liver weight.	Gavage with 50 mg/mouse/day; 50 mg DiR-labelling EVs or 50mg PKH67-labelling EVs for imaging
Mingzhen Zhang et al. 2017	Ginger	Ginger juice centrifugated at 3,000g for 20min, 10,000g for 40min, 150,000g for 2h and the pellet was resuspended in PBS and sonicated. Then spun at 150,000g for 2h in a sucrose gradient (8%/30%/45%). Band 2 between 30%/45% was selected as EVs.	232.7nm	polydispersity index=0.163	Phosphatidic acid (41.9% of total lipids), digalactoyldiacylglycerol (27.4%), monogalactosyldiacylglycerol (18.9%)	ND	ND	ND	Use of lipid EVs encapsulate and transfer siRNA in RAW 264.7 and colon-26 cells	Mice were given siRNA-EVs orally (twice at 12h interval) to knock-down CD98 specifically in the ileum and colon
Zhehao Zhao et al. 2018	coconut water or milk coconut	Fresh coconut water or milk samples were centrifuged 3 times at 4 °C for 30 min each at 300g, 1500g, and 7000g to remove cells, large debris, and fat, respectively. The supernatant was prepared by ultracentrifugation (Avanti J-25 and Optima L-100XP, Beckman Coulter, Brea, CA, U.S.A.) at 50000g, 70000g, 100000g, and 130000g for 1 h, followed by filtration through 0.22 µm filters	coconut water : 59.72 nm milk: 100.40 nm	ND	ND	ND	ND	ND	ND	ND
Qilong Wang et al. 2013	grapefruit, tomatoes, grape		Grape: 1.76 ± 0.15 g/kg Grapefruit: 2.21 ± 0.044 g/kg Tomatoes : 0.44 ± 0.02 g/kg	ND	ND	ND	ND	ND	ND	ND
Zhongbin Deng et al. 2017	Broccoli	Broccoli juice was sequentially centrifugated at 1,000g (10mn), 3,000g (20mn), 10,000g (40mn), 100,000g (90mn). The resuspended EV pellet was further purified with Biomax-500 column filtration and sucrose gradient	32.4nm	-39.2 to -2.62 mV	Presence of sulforaphane	ND	ND	Dendritic cells (DCs)	EVs protect mice against colitis by activating AMPK in DCs. Broccoli EV's effects are dependant from sulforaphane contained in EVs	Mice were given EVs orally (250 µg/mouse in PBS, labeled with PKH26) before (every day for 10d) and after (every 2 days) induction of DSS-induced colitis
Juan Xiao et al. 2018	11 edible fruits and vegetables	differential centrifugations	from 100 to 1000nm						418miRNAs (from 32 to 127 miRNAs per species)	