

Arugula nanovesicles counteract diet-induced obesity and modulate gut inflammation and microbiota *in vivo*

Supplementary information

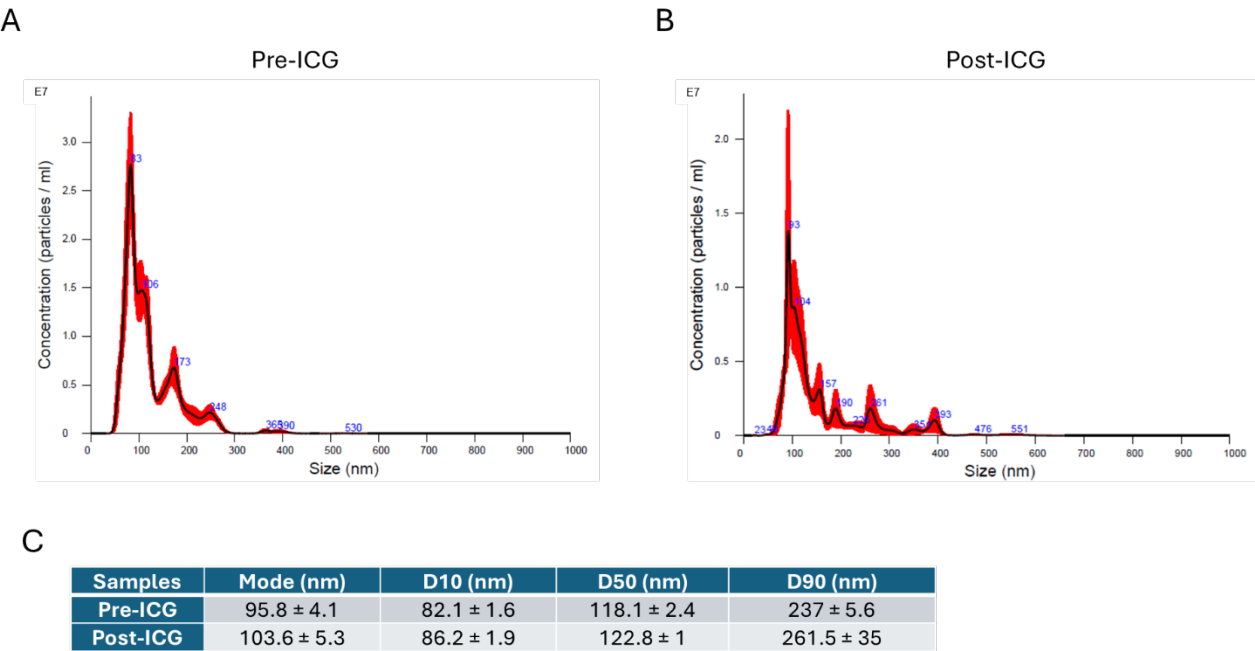


Figure S1– Dimensional analysis of ALVs before and after ICG loading. (A) Nanoparticle tracking analysis (NTA) profile of ALVs prior to ICG loading. (B) NTA profile of ALVs following ICG loading. (C) Table summarizing dimensional parameters of ALVs, including mode size and percentiles D10, D50, and D90. D10 corresponds to the diameter below which 10% of the particles are distributed, D50 represents the median particle size, and D90 indicates the diameter below which 90% of the particles fall.

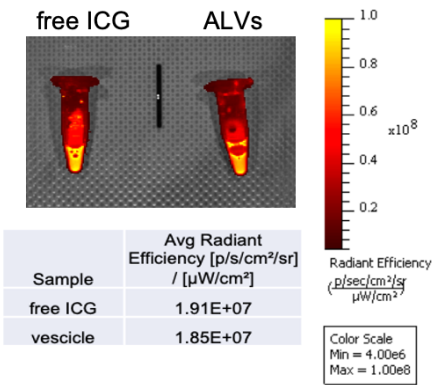


Figure S2 – Fluorescence analysis of samples used for the *in vivo* biodistribution: free ICG (left), ALVs (right). The picture shows the comparison of the fluorescence as acquired with an IVIS Spectrum imaging system equipped with appropriate filters to detect the ICG signal. The intensity of the fluorescence signal (showing an average radiance efficiency of $1.9\text{E}7 \text{ [p/s/cm}^2\text{/sr]} / \text{[}\mu\text{W/cm}^2\text{]}$ in both the samples) is represented in pseudocolors according to the reported scale bar.

Table S1 – HFHF diet composition



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10008805 108805 C1088-05 NASH high fructose + palm oil(w/40%fat, w/20%fructos, 2%chol)

Nr.	Inhaltsstoff	Einheit	Bedarf	Gehalt	Differenz
1	Rohprotein / Crude Protein	mg/kg		170782,922	
2	Rohfett / Crude Fat	mg/kg		220285,971	
3	Rohfaser / Crude Fibre	mg/kg		55382,504	
4	Rohasche / Crude Ash	mg/kg		54653,404	
5	Feuchtigkeit / Moisture	mg/kg		50681,246	
6	Monosaccharide(s)	mg/kg		200514,949	
7	Disaccharide(s)	mg/kg		7499,250	
8	Polysaccharide(s)	mg/kg		195270,473	
9	Umsetzb. Energie/Metab. Energy	kcal/kg		4350,650	
10	Lysin / Lysine	mg/kg		17340,381	
11	Methionin / Methionine	mg/kg		10647,435	
12	Cystin / Cystine	mg/kg		3153,995	
13	Threonin / Threonine	mg/kg		7078,807	
14	Tryptophan	mg/kg		1964,124	
15	Arginin / Arginine	mg/kg		9731,832	
16	Histidin / Histidine	mg/kg		5218,783	
17	Isoleucin / Isoleucine	mg/kg		7145,475	
18	Leucin / Leucine	mg/kg		14520,763	
19	Phenylalanin / Phenylalanine	mg/kg		7072,908	
20	Valin / Valine	mg/kg		3201,810	
21	Alanin / Alanine	mg/kg		2369,763	
22	Asparaginsäure / Aspartic acid	mg/kg		3449,285	
23	Glutaminsäure / Glutamic acid	mg/kg		23297,785	
24	Glycin / Glycine	mg/kg		3056,694	
25	Prolin / Proline	mg/kg		12577,652	
26	Serin / Serine	mg/kg		5164,584	
27	Tyrosin / Tyrosine	mg/kg		9198,375	
28	Vitamin A	I.E./kg		14998,500	
29	Vitamin D3	I.E./kg		499,950	
30	Vitamin E	mg/kg		150,385	
31	Vitamin K3 als/as Menadion(e)	mg/kg		9,999	
32	Vitamin B1	mg/kg		20,038	
33	Vitamin B2	mg/kg		20,320	
34	Vitamin B6	mg/kg		15,032	
35	Vitamin B12	mg/kg		0,041	
36	Nikotinsäure / Nicotinic acid	mg/kg		50,165	
37	Pantothenre./Pantothenic acid	mg/kg		50,101	
38	Folsäure / Folic acid	mg/kg		10,00140	
39	Biotin	mg/kg		0,201	
40	Cholinchlorid/Choline chloride	mg/kg		1011,399	
43	Inosit / Inositol	mg/kg		110,989	
44	Vitamin C	mg/kg		20,998	
45	Calcium	mg/kg		9003,870	
46	Ges.Phosphor / Phosphorus	mg/kg		7419,577	
47	Verd.Phosphor/Digest.Phosphorus	mg/kg		7135,737	
48	Magnesium	mg/kg		663,354	
49	Natrium / Sodium	mg/kg		2419,622	
50	Kalium / Potassium	mg/kg		7020,346	
51	Schwefel / Sulfur	mg/kg		2742,749	
52	Chlor / Chlorine	mg/kg		3639,636	

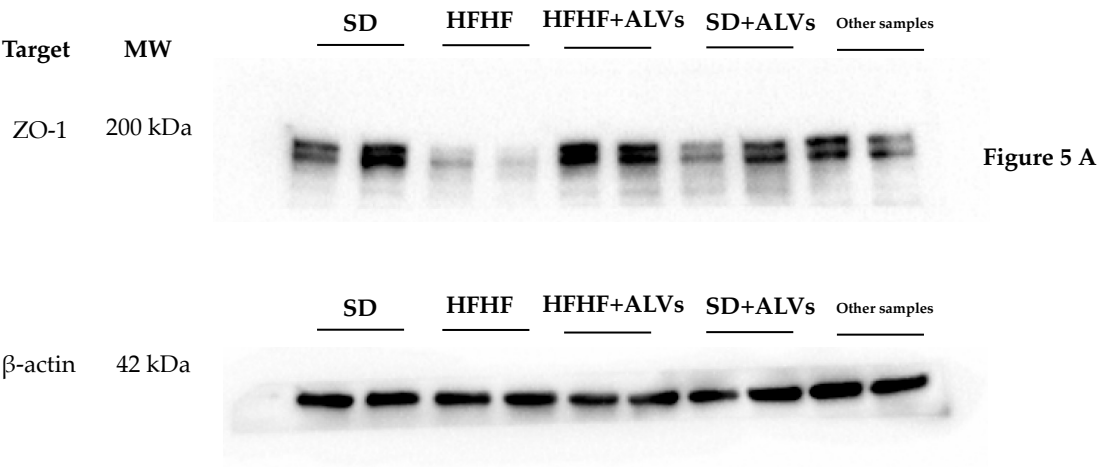
Table S2 – Average of food consumption for each experimental group.

	SD	SD+ALVs	HFHF	HFHF+ALVs
Daily food intake (g)	3.41 ± 0.18	3.36 ± 0.15	3.38 ± 0.25	3.49 ± 0.15

ALVs: Arugula leaves vesicles; SD: standard diet; HFHF: high fat high fructose diet; SD+ALVs: standard diet + Arugula leaves vesicles; HFHF+ALVs: high fat high fructose diet + Arugula leaves vesicles.

Supplementary Data 1 – Overview of protein corona identified around ALVs by Shotgun Proteomics Analysis.

Original Western Blot membranes pictures



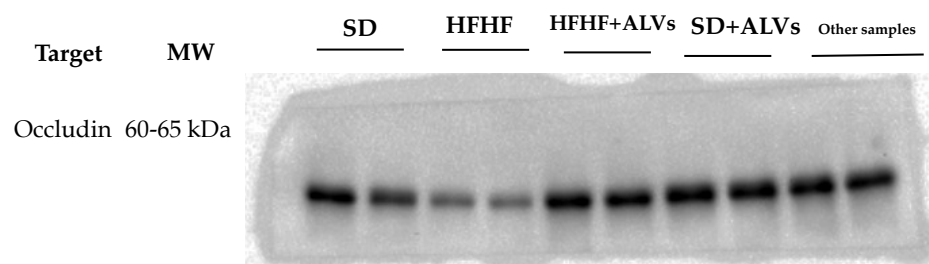


Figure 5 B

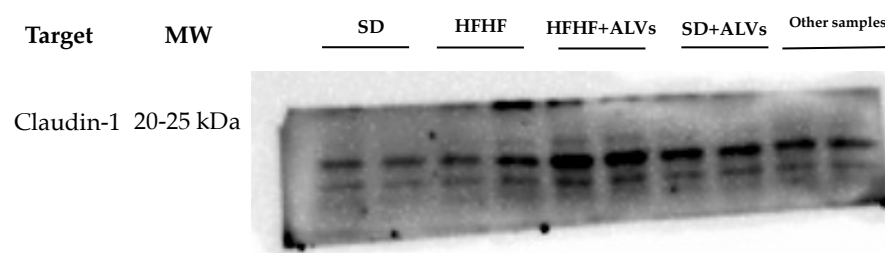
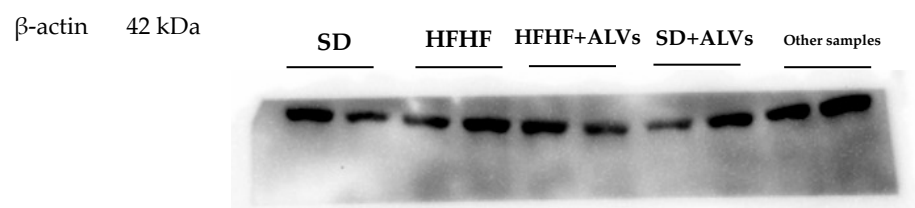
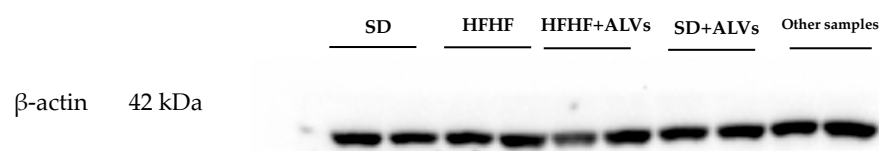


Figure 5 C



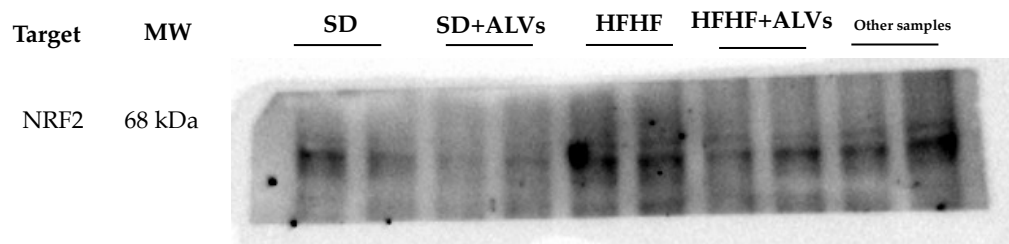


Figure 6 A

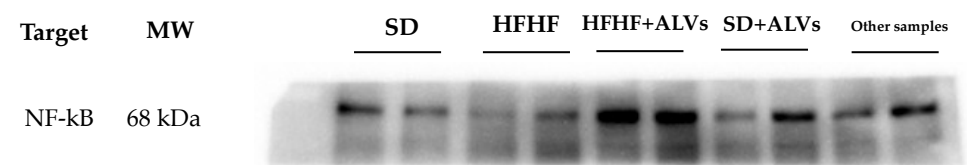
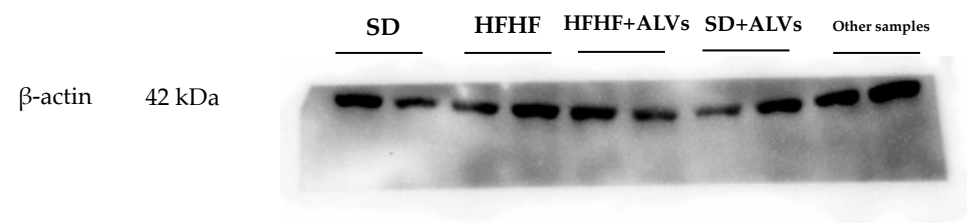
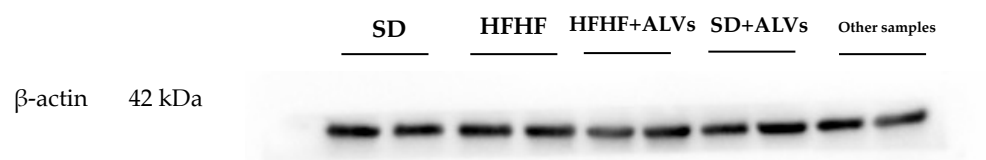


Figure 6 B



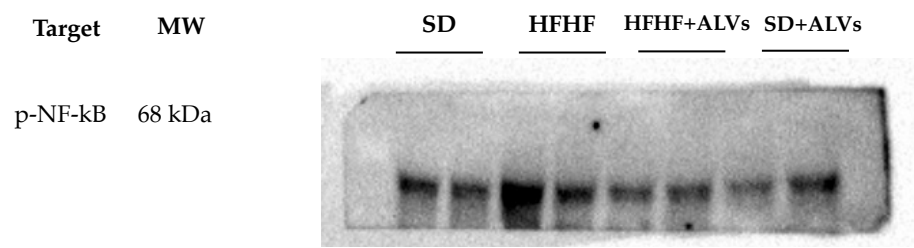


Figure 6 C

