Akkermansia muciniphila derived extracellular vesicles influence gut permeability through regulation of tight junctions

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Supplementary Figure S1

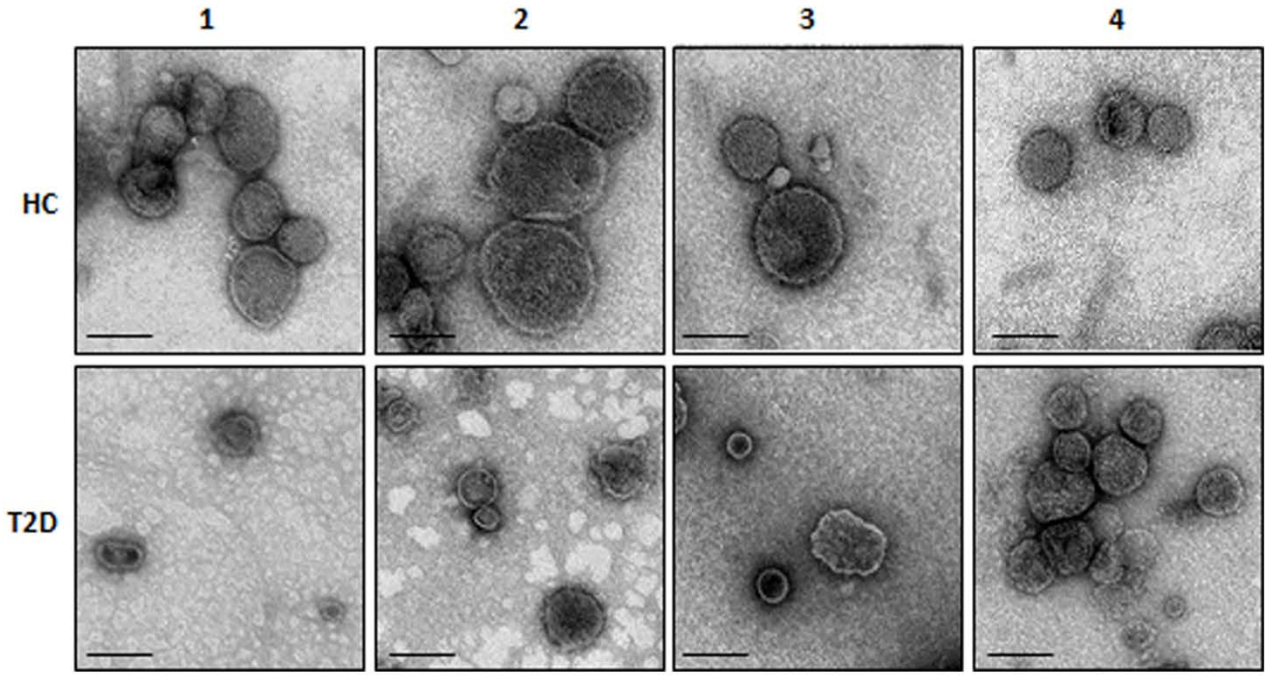

Figure S1: Comparison of fecal EV from T2D patients with Healthy controls.

Transmission electron microscopic (TEM) images demonstrating the spherical shape of EVs derived from healthy controls and T2D patients, showing healthier AmEVs in healthy controls compared to T2D patients. Scale bar is 200 nm. HC, Healthy control. T2D, type 2 diabetes pateints.

Supplementary Figure S2

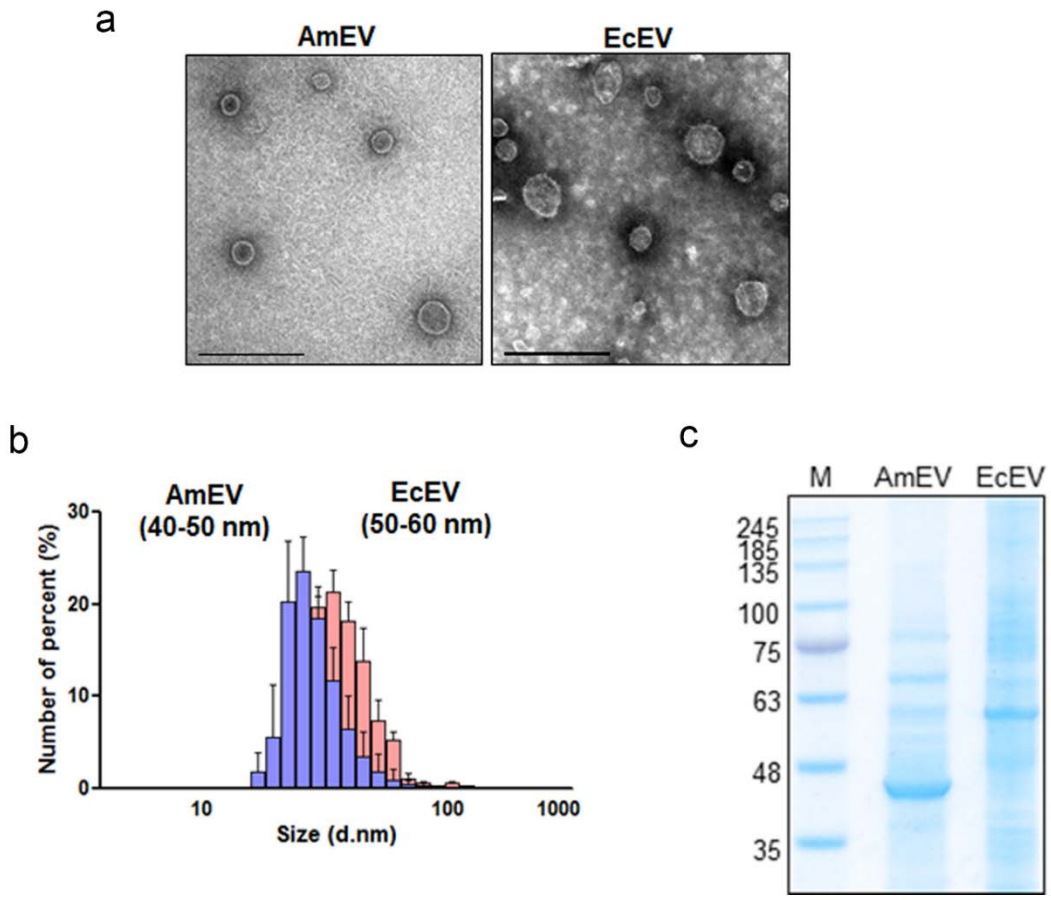

Figure S2. Isolation and characterization of *A. muciniphila* derived EVs.

(a) Transmission electron microscopic (TEM) images demonstrating the spherical shape of *A. muciniphila*-derived EVs (AmEVs) and *E. coli*-derived EVs (EcEVs). Scale bar is 200 nm. **(b)** EV size (d.nm) was measured using dynamic light scattering (DLS). Both EVs had similar diameter lengths. **(c)** Protein profiles of EVs by sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS-PAGE) analysis. Each EV exhibited different protein band patterns.

Supplementary Figure S3

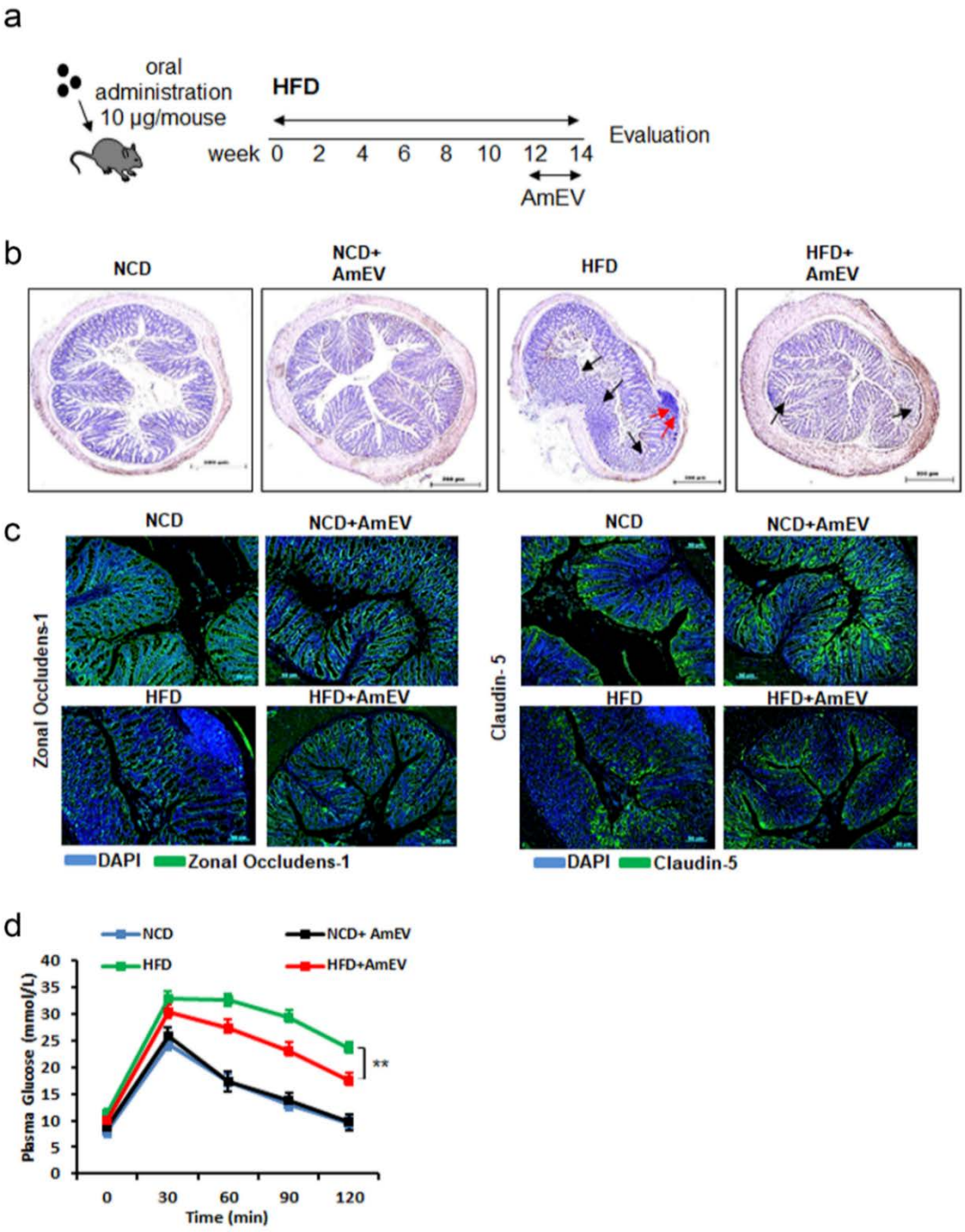

Figure S3. AmEV feeding improves metabolic features in HFD fed mice

(a) Schematic representation of the time scale of HFD feeding and AmEV feeding in C57BL/6 mice. **(b)** Hematoxylin and Eosin staining of colon sections from NCD, NCD+ AmEV, HFD and HFD+ Am EV fed mice shown at 4x magnification. Black arrows indicate damage to the intestinal epithelial layer and recruitment of immune cells into the intestinal epithelial layer. Red arrows indicate the immune cell recruitment. Scale bar 200μm. **(c)** Immunohistochemistry images showing zonal occludems-1 and claudin 5 expression in NCD, NCD + AmEV, HFD, HFD+ AmEV fed animals. Scale bar 50μm. **(d)** Glucose tolerance test in NCD, NCD+ AmEV, HFD and HFD+ AmEV fed mice. Animals were given NCD or HFD for 10 weeks followed by oral gavaging of 10μg of AmEV/mouse for 2 weeks. The animals were fasted 16h followed by intra-peritoneal injection of 1g/kg glucose. Blood glucose level was measured using a blood glucose kit at indicated time points. All data are presented as the mean ± SEM of 3 experiments performed in triplicates; n=5-7 per group; * indicates $p < 0.05$, ** indicates $p < 0.01$.

Supplementary Figure S4

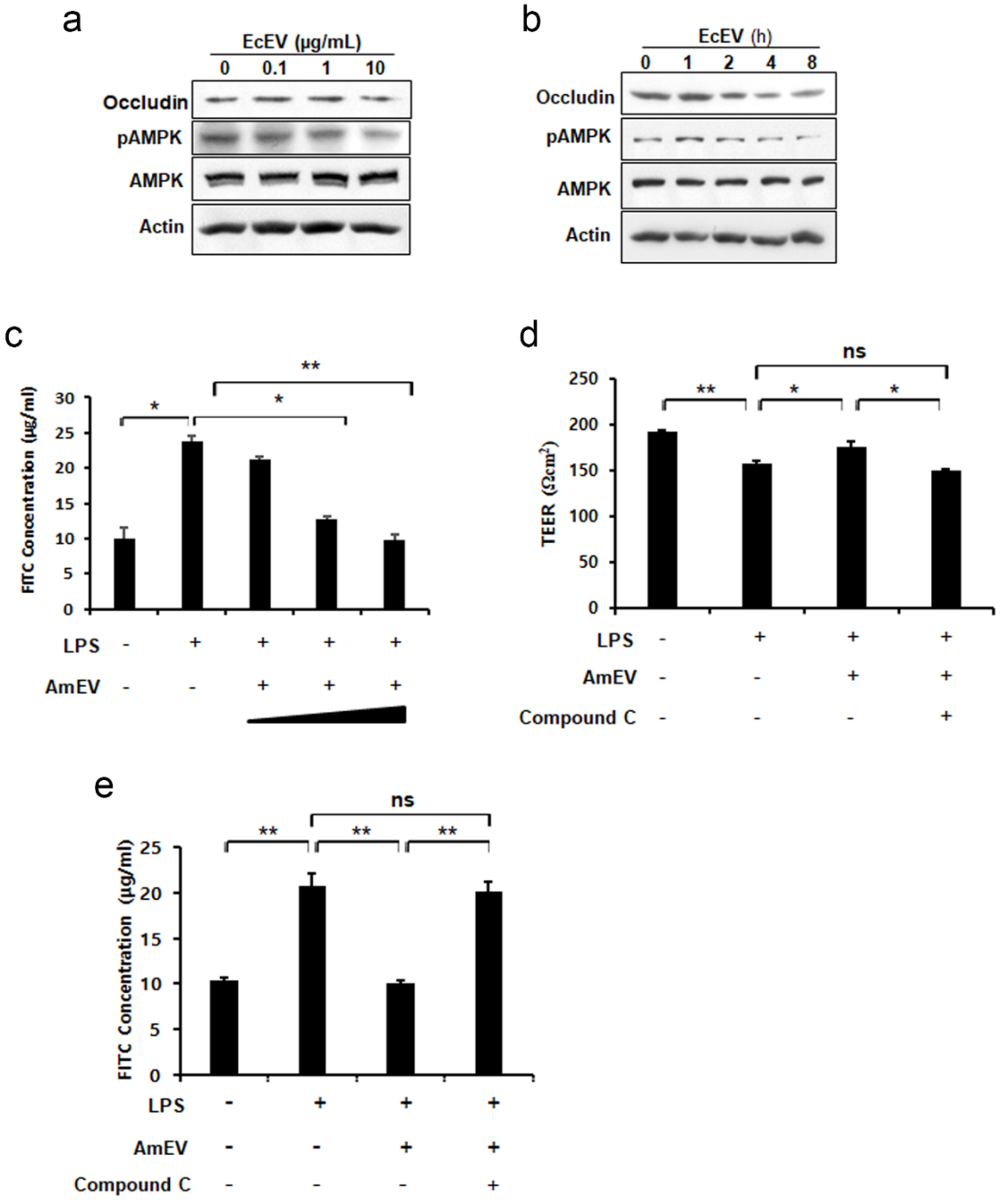

Figure S4: Only AmEV, and not EcEV can improve intestinal permeability

(a) Western blot image showing the expression of occludin, pAMPK and total AMPK in Caco2 cells after dose dependent treatment of EcEV. Caco2 cells were treated with 0.1µg/ml, 1µg/ml and 10µg/ml of EcEV and expression of occludin, p-AMPK, total AMPK and actin was analyzed after 4 hours by immune blotting. **(b)** Time dependent changes in occludin, pAMPK and total AMPK expression after EcEV treatment in Caco2 cells. . Caco2 cells were treated with 1µg/ml of EcEV and expression of occludin, p-AMPK, total AMPK and actin was analyzed after 4 hours by immune blotting. **(c)** AmEV treatment improves LPS induced intestinal permeability in a dose dependent manner. Caco2 monolayer was treated with LPS, or LPS + AmEV at concentrations 0.1µg/ml, 1µg/ml, and 10µg/ml. *In-vitro* permeability was measured using FITC-Dextran assay. **(d)** Caco2 cells were pre-treated with AMPK specific inhibitor for 30 min, after which cells were treated with LPS or LPS+ AmEV. TEER resistance was measured using volt-ohm meter (Merck Millipore). **(e)** Caco2 monolayer transwell was pre-incubated with AMPK- specific inhibitor compound C for 30 min, after which cells were treated with LPS (5µg/ml) or LPS (5µg/ml) + AmEV (1µg/ml) as indicated. 4 hours later the upper chamber of transwell was treated with 1mg/ml of FITC-Dextran and the fluorescence in the lower chamber was measured after 4 hours using fluorescence spectrometer. All data are presented as the mean ± SEM of 3 experiments performed in triplicates; * indicates $p < 0.05$, ** indicates $p < 0.01$.

Supplementary Table S1

Subjects	Sex	Age	Date of diagnosis	HbA1c (%)
HC 1	M	27		
HC 2	M	28		
HC 3	M	28		
HC 4	M	30		
HC 5	F	42		
HC 6	M	58		
HC 7	F	63		
HC 8	M	65		
T2D 1	M	22	2007	11.1
T2D 2	M	47	2011	15.4
T2D 3	M	48	1999	9.2
T2D 4	F	48	2003	11.3
T2D 5	F	49	1996	10.4
T2D 6	M	50	2000	9.4
T2D 7	M	51	1998	11.2
T2D 8	M	51	2003	12.2
T2D 9	F	52	1995	11.6
T2D 10	F	52	2001	10.7
T2D 11	M	53	2004	10.1
T2D 12	F	54	1977	8.5

HC, healthy control; T2D, type 2 diabetes; HbA1c, glycosylated hemoglobin

Supplementary Table 1: Age, Sex and disease diagnosis details of type 2 diabetic patients. Details of healthy controls are also given.