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Supplemental Information

Use of Nanovesicles from Orange Juice to Reverse Diet-Induced Gut Modifications in Diet-Induced Obese Mice

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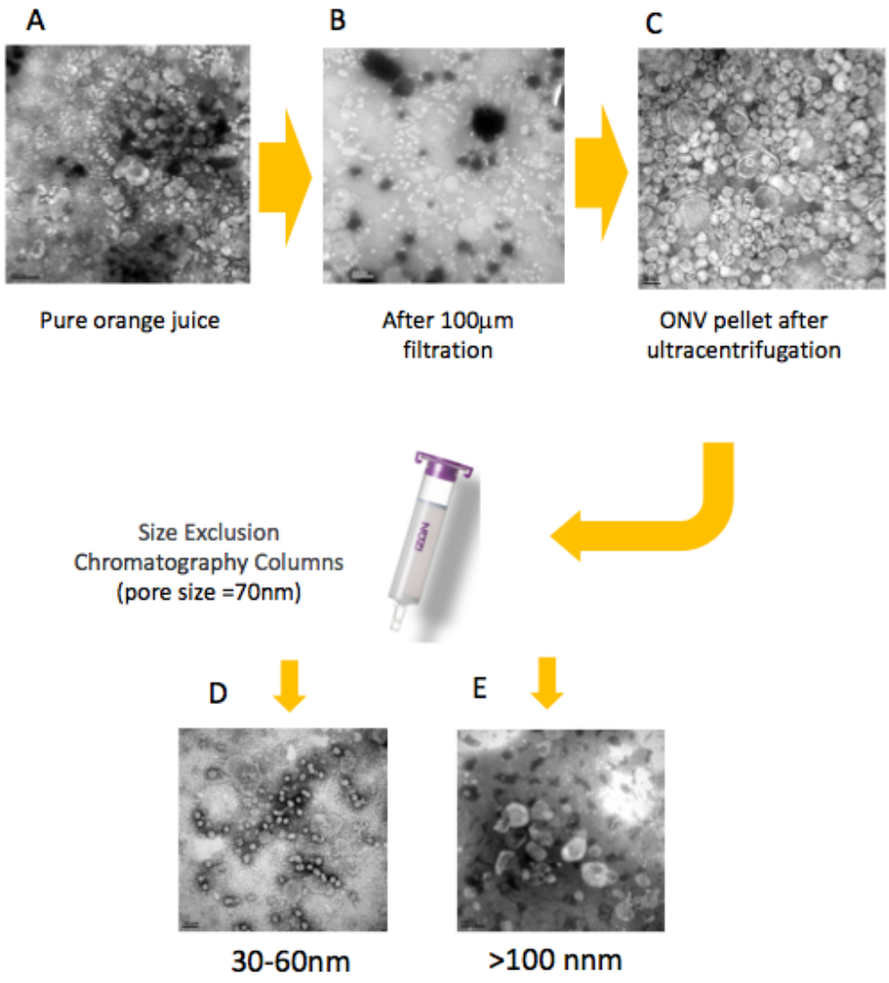

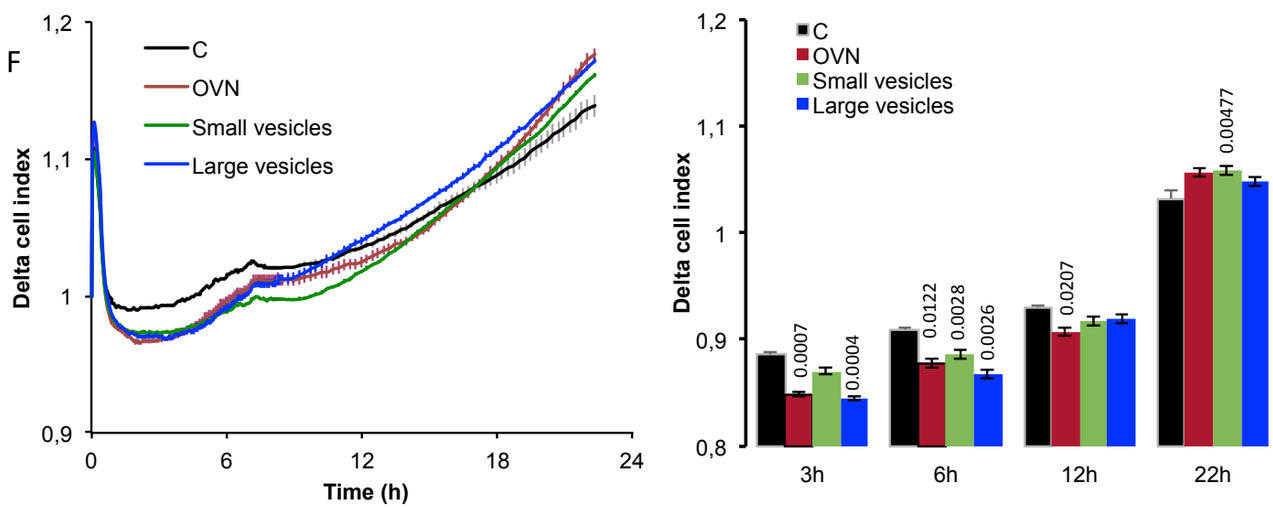

Figure S1: Transmission Electron Microscopy (TEM) images from: (A) pure orange juice; (B) after the first step of orange juice filtration at 100 µm; (C) from the pellet after ultracentrifugation and resuspension in PBS. (Bar=200nm). (D and E) ONV pellet re-suspended in PBS was further passed through a size exclusion column (IZON) and fractions containing proteins were visualized by TEM (Bar=100nm). (F) The activities of the small (D) and large vesicles (E) was tested independently on Caco-2 cells by using the xCELLigence live cell analysis System. Caco-2 cells were plated on 96-wells E-plates at 2500 cells/well in DMEM/10% FBS for 3 days then maintained in DMEM/5% FBS exosome-free for 2 hours before treatment with 2 µg/ml of either small or large vesicles, or ONV. The left panel represents real-time monitoring of the cell index substracted from the cell index at T=0 (mean delta cell index, every 5 min during 50 cycles then every 15 min) for 22 h. The right panel represents the slopes at T=22h (mean values +/- sem) (n=4 to 6 replicates), with significant p-values (*=p<0.05, each condition vs untreated caco-2).

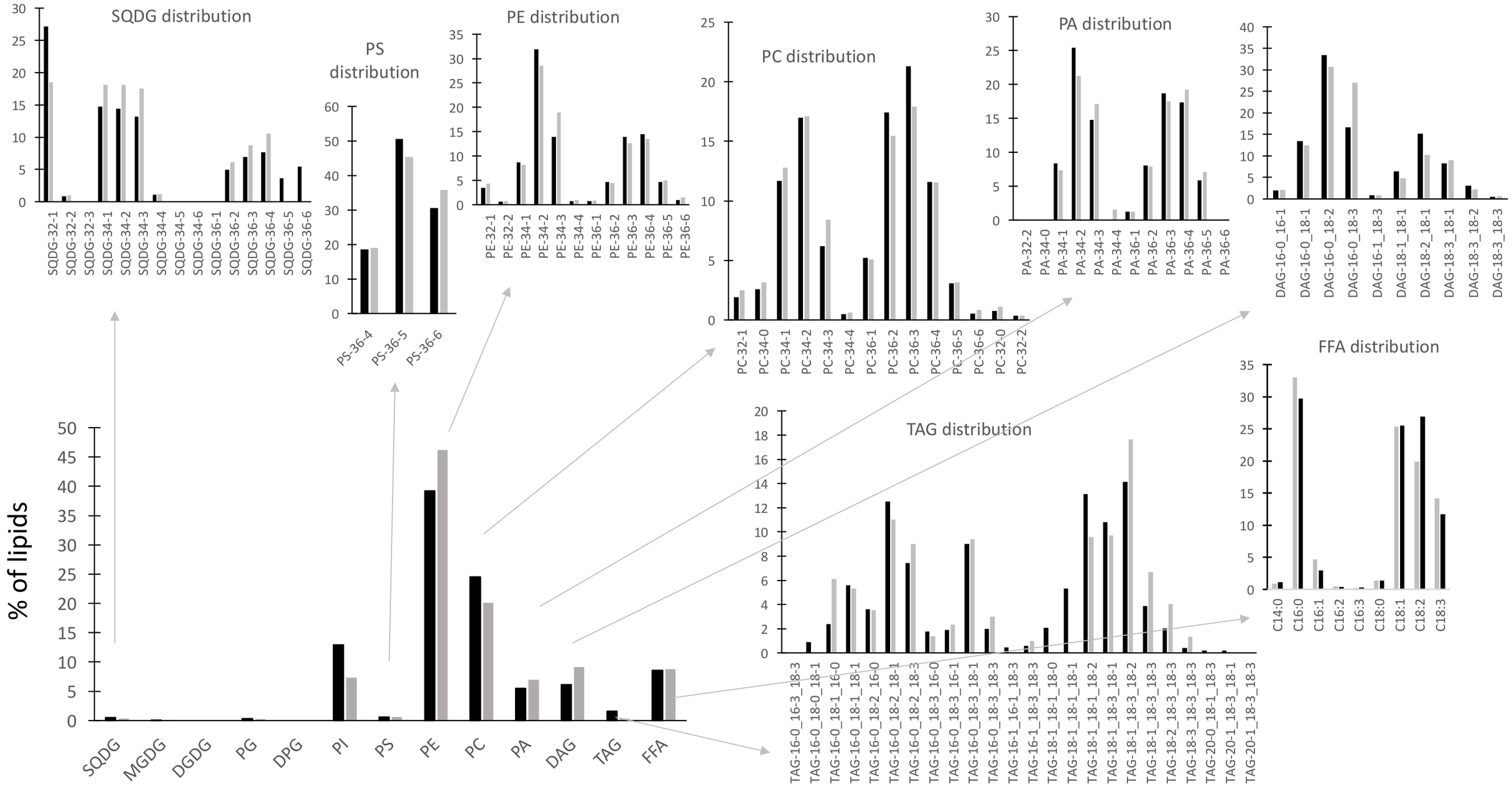

Figure S2: Lipidomic analyses from 1mg ONV. The two colors represent two independent lipidomic analyses from 2 independent ONV preparations

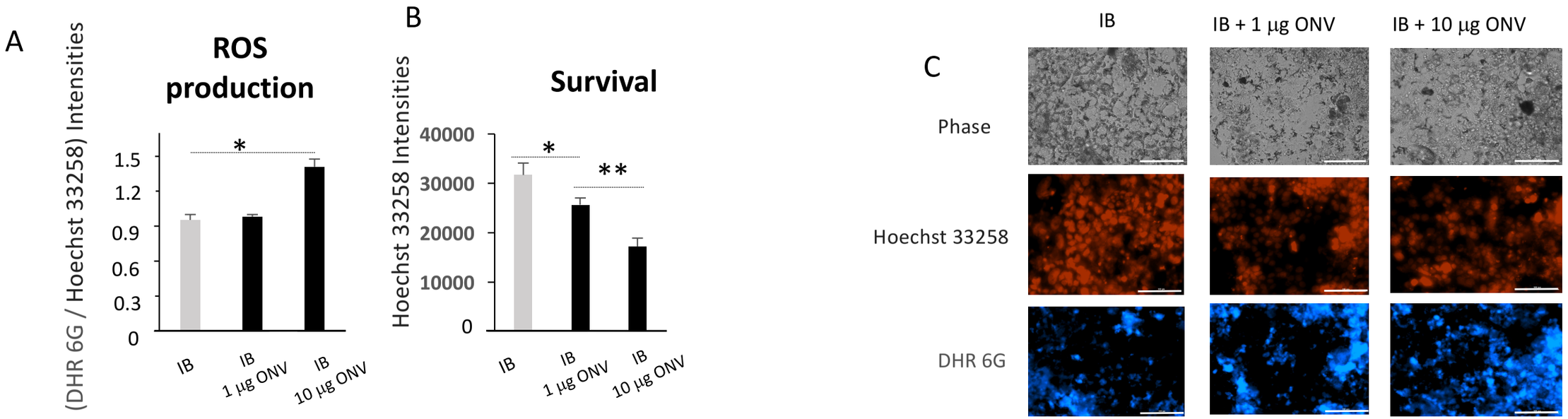

Figure S3: (A) Reactive oxygen species (ROS) production (DHR 6G fluorescence) in IB treated with different ONV concentrations. (B) Quantification of cell death in IB treated with different quantities of ONV.
Results represent Hoescht 33258 fluorescence intensities. (C) Representative images used to quantify the number of nuclei (Hoescht 33258 fluorescence) and the ROS production (DHR 6G fluorescence) in the IB treated with ONV. Bar=100µm.

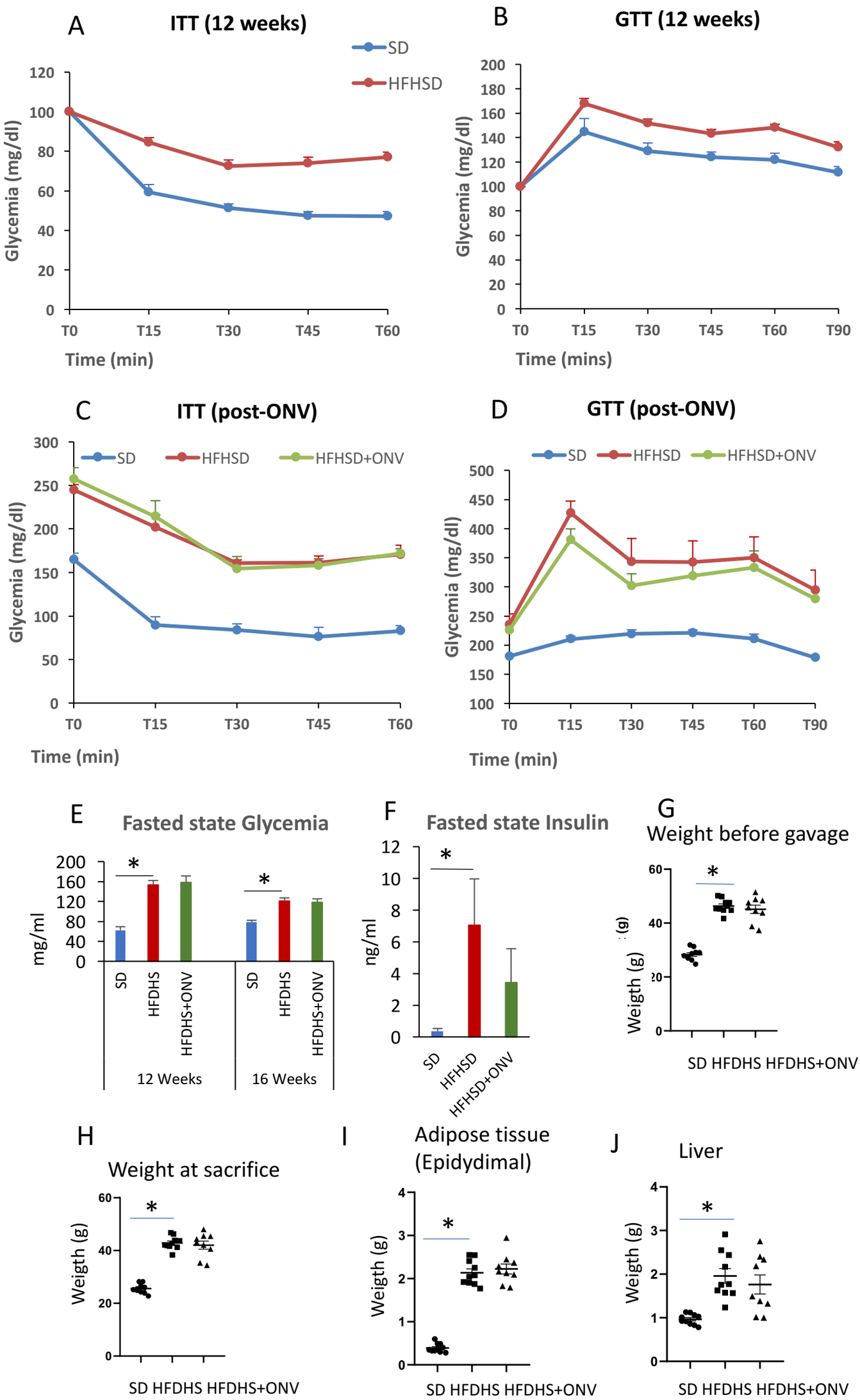

Figure S4: ONV effects in DIO mice. (A) Insulin tolerance test and (B) Glucose tolerance test at 12 weeks before the gavage with ONV (n=10). (C) Insulin tolerance test and (D) Glucose tolerance test after one month of gavage with 150µg ONV (n=5). (E) Glycemia before and after gavage (n=10), (F) Plasma insulin after gavage (n=3), (G and H) body weight before and after gavage (n=10). (I) Epidydimal adipose tissue weight at sacrifice (n=10), (J) Liver weight at sacrifice (n=10).