Panel S3: PROTEIN EXTRACTION AND WESTERN BLOT ANALYSIS

An equal amount of snap frozen liver sample (200 mg) were lysed in 800 µl of RIPA buffer (Sigma-Aldrich), supplemented with a protease and phosphatase inhibitor cocktail (Sigma-Aldrich) together with 0.5 mm zirconium oxide beads (Next Advanced, NY). The homogenization of tissue was performed using a Bullet Blender (Next Advanced, NY) at a speed of 8 rpm for three minutes at 4°C. Then, the homogenized tissue was centrifuged at 12,000 g for ten minutes at 4°C and the proteins supernatant was recovered. Proteins were quantified by Bradford method and 50 µg of proteins for each sample were run by electrophoresis on 4–20% precast gel (Biorad, USA) under reducing conditions and blotted onto PVDF membrane using the Trans-Blot Turbo Transfer System (Biorad, USA). Membranes were blocked with 10% milk-PBS-Tween 0,1% for 1 hour at RT and then incubated with the AbCam primary antibody overnight at 4°C: E-Selectin (ab18981), P-Selectin (ab59738), V-CAM1 (ab134047) and GAPDH (ab8245). After three washing in PBS-Tween 0,1%, the membrane were incubated with the appropriated secondary antibody HRP (Invitrogen, Thermofisher), then washed three times. After the addition of ECL substrate, the signal was revealed by chemiluminescence. The images were acquired on Chemidoc and analysed with ImageLab (both from Biorad, USA). The protein level was expressed as the ratio between the protein of interest and the endogenous control GAPDH.