

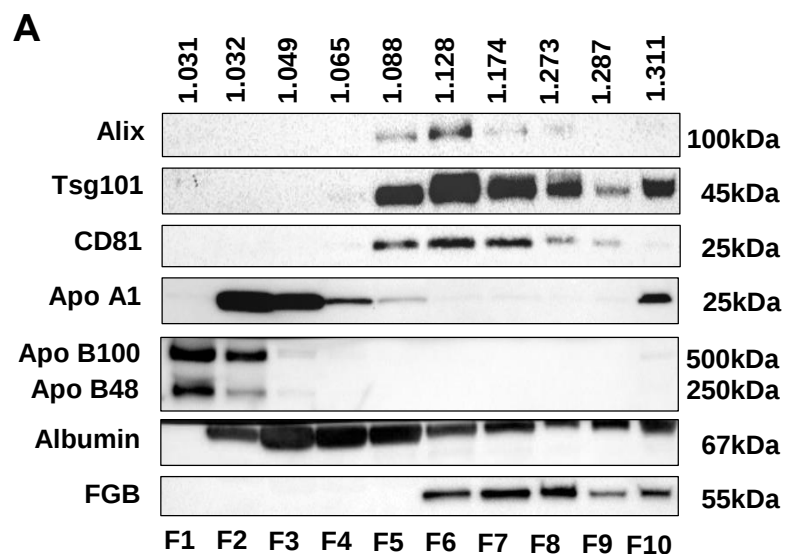
Supplementary Material

Isolation of high-purity extracellular vesicles by the combination of iodixanol density gradient ultracentrifugation and bind-elute chromatography from blood plasma

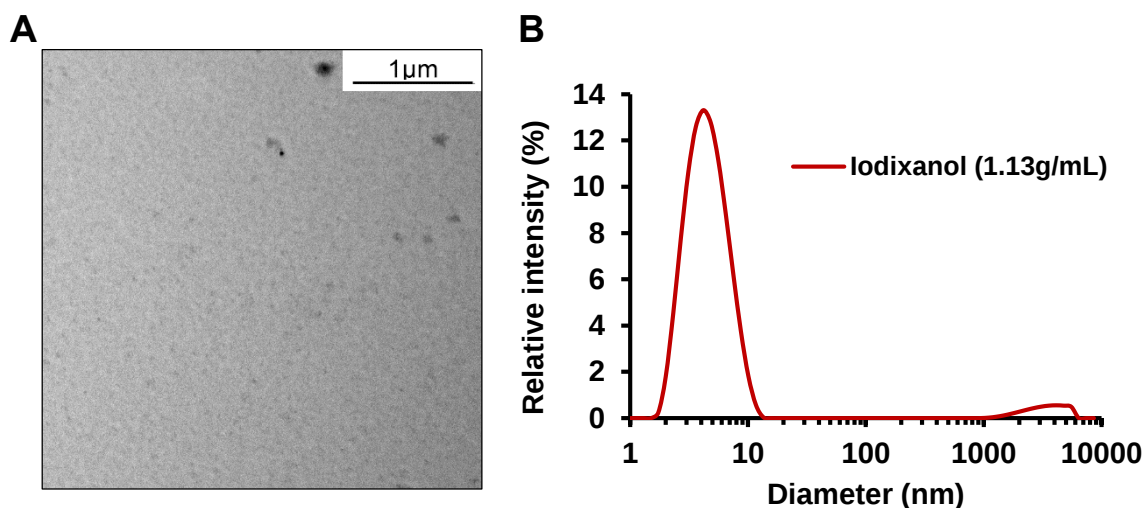
Zsófia Onódi¹, Csilla Pelyhe¹, Csilla Terézia Nagy¹, Gábor B. Brenner¹, Laura Almási¹, Ágnes Kittel², Mateja Manček-Keber^{3,4}, Péter Ferdinandy^{1,5}, Edit I. Buzás⁶, Zoltán Giricz^{1,5, *}

*** Correspondence:**

Corresponding author: Zoltán Giricz PharmD PhD
address: H-1089 Budapest, Nagyvárad tér 4. Hungary
e-mail: giricz.zoltan@med.semmelweis-univ.hu
Tel: +3612104416
Fax: +3612104412

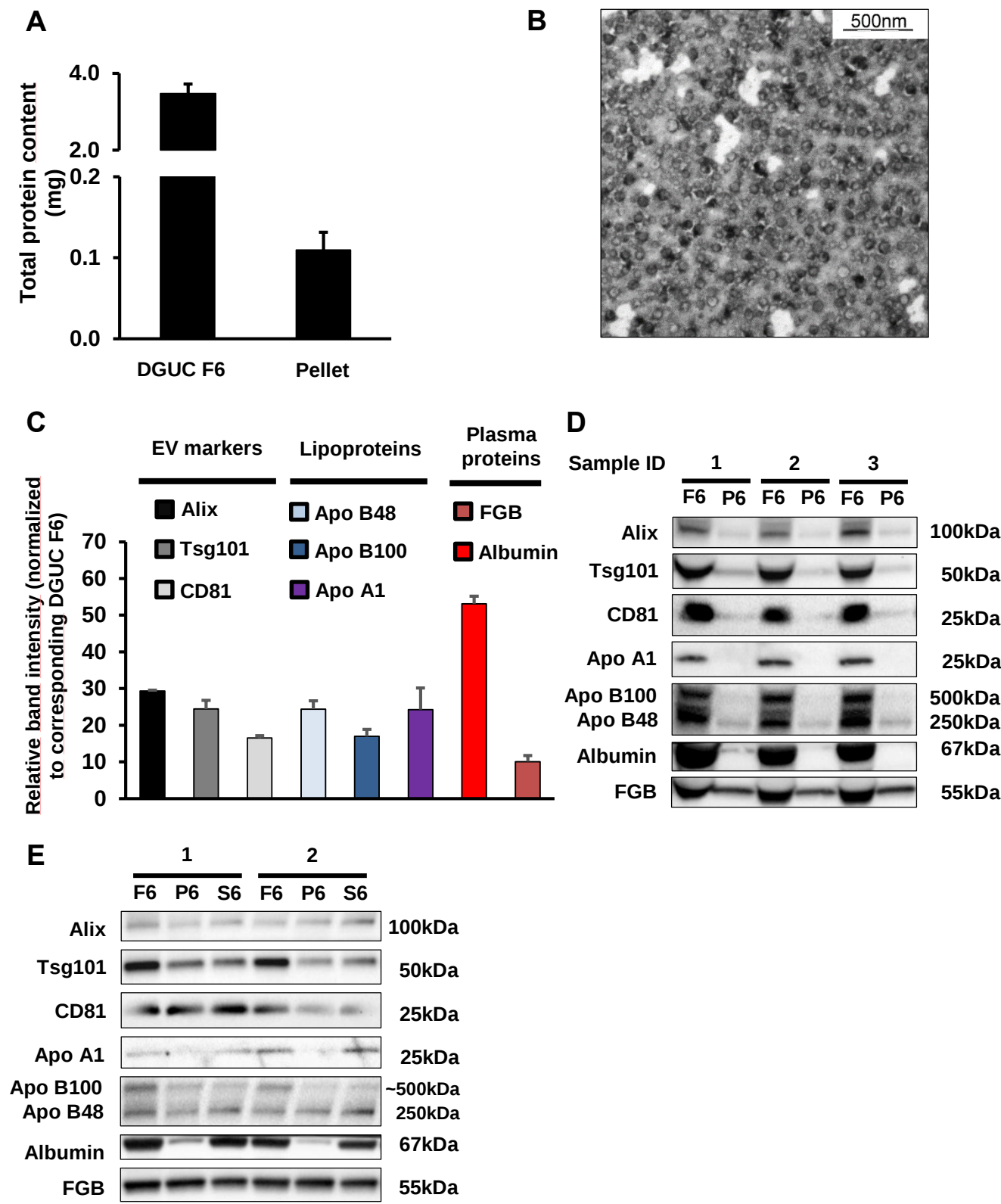
Supplementary Figure 1

Supplementary Figure 1 - Representative images from equal protein (10 μ g) loading Western blot from small-scale DGUC fractions. DGUC: density gradient ultracentrifugation; FGB: fibrinogen beta chain

Supplementary Figure 2

Supplementary Figure 2 - Analysis of 1.13mg/mL iodixanol solution. Representative electron microscopy image, sample prepared by grid-adsorption method (A) and dynamic light scattering measurement (B) from control iodixanol solution (~20 w/V%, 1.13g/mL)

Supplementary Figure 3

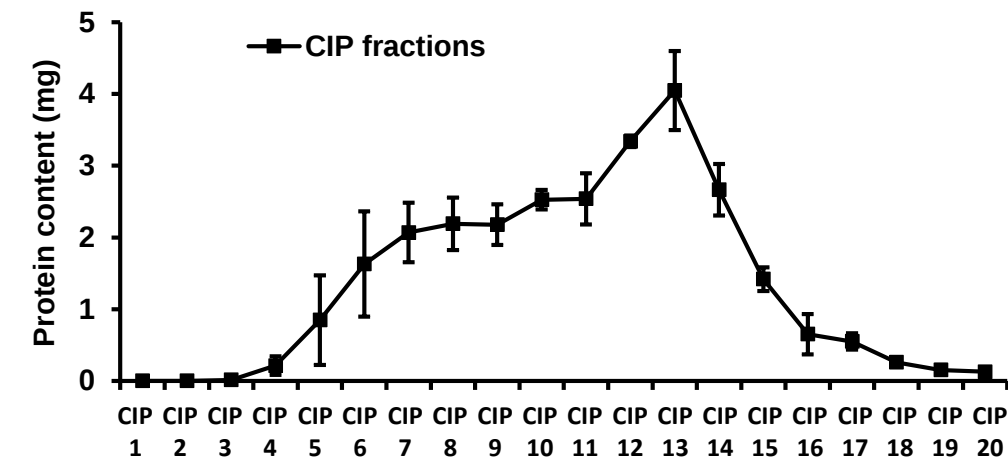


Supplementary figure 3 – Analysis of 3h ultracentrifugation of small-scale DGUC F6. Average of protein content (A), representative electron microscopy image, sample prepared by grid-adsorption method (B) and evaluation of Western blots (C) of 3h UC pellet (P6) compared to corresponding DGUC F6. Evaluation (C) and representative images (D) from Western blot comparison of F6 to P6. Equal protein loading Western blot of F6,

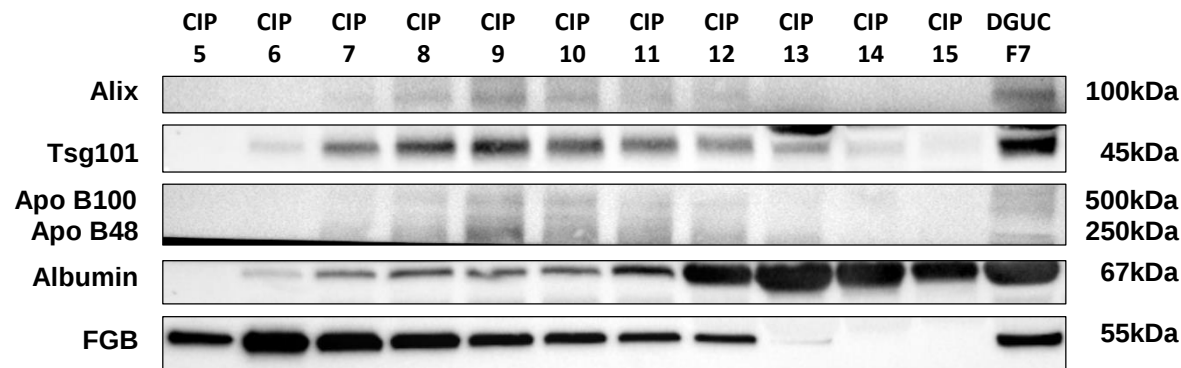
P6 and S6 (D; n=3). Data are expressed as mean $\pm$ standard error of mean. DGUC: density gradient ultracentrifugation; F: fraction; P6: pellet from F6; S6: supernatant from F6; FGB: fibrinogen beta chain

Supplementary Figure 4

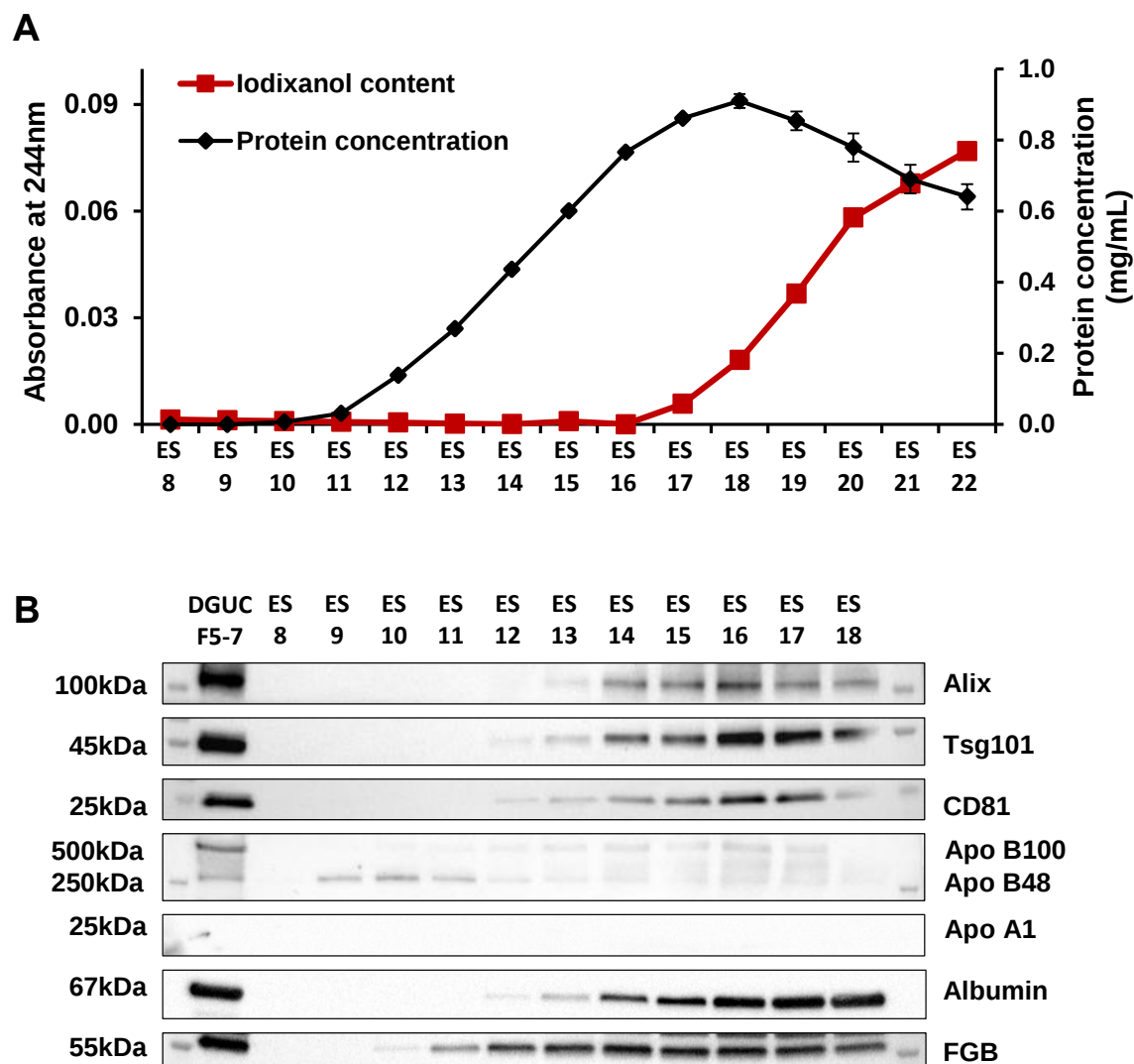
A



B

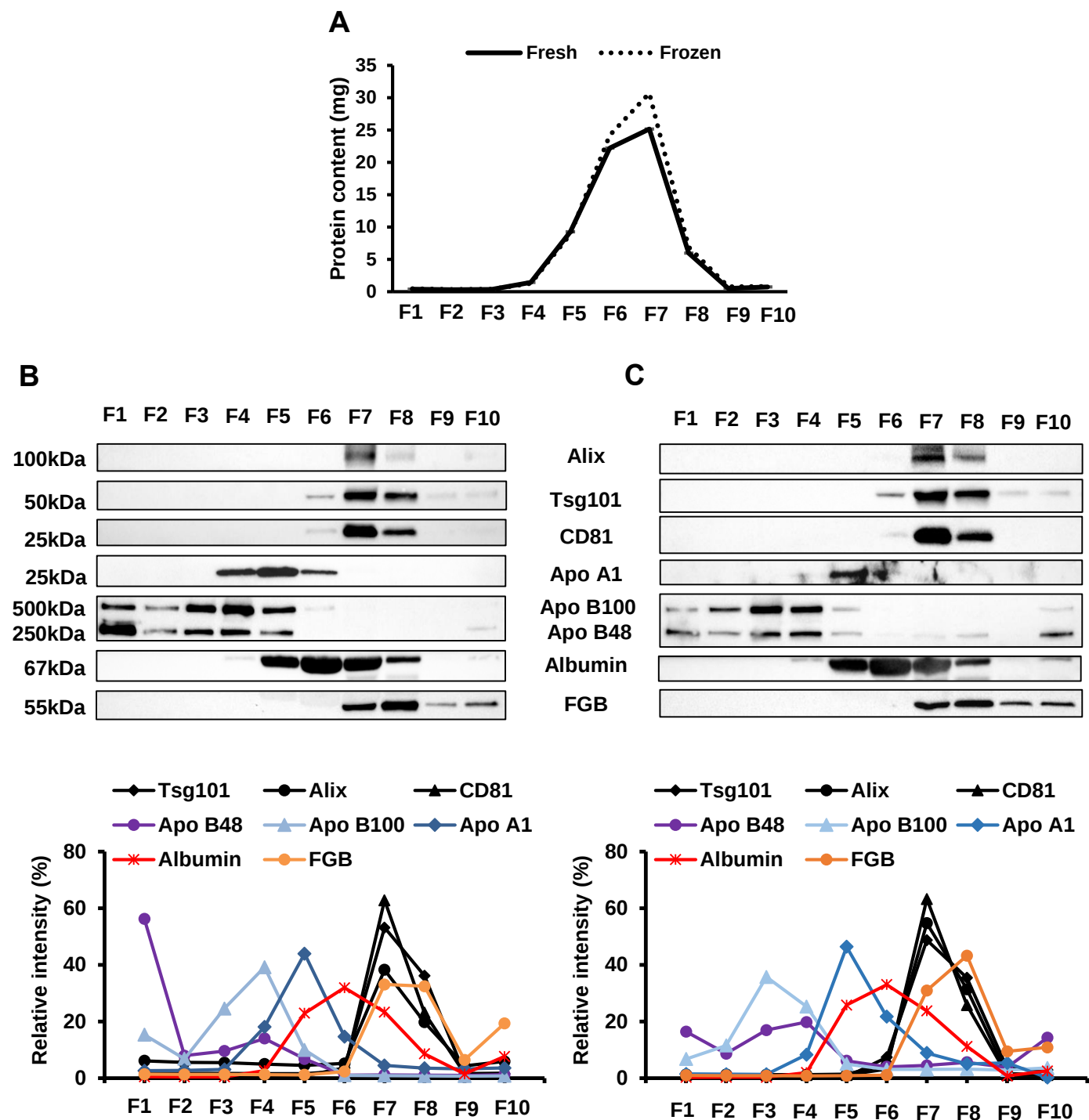


Supplementary figure 4 – Analysis of eluted CIP fractions from Capto Core 700 column. Average of protein content (A) of eluted CIP fractions. Representative Western blot images from CIP5-15 and DGUC F7 (B; n=2). Data are expressed as *mean ± standard error of mean*. CIP: cleaning-in-place; DGUC: density gradient ultracentrifugation; F: fraction; FGB: fibrinogen beta chain

Supplementary Figure 5

Supplementary figure 5 – Analysis of Exo-Spin™ column-based EV-isolation from small-scale DGUC F5-7. Average of protein content (A) and representative Western blot images (B) of Exo-Spin™ isolation compared to corresponding DGUC F5-7. Data are expressed as mean $\pm$ standard error of mean. ES: Exo-Spin fraction; DGUC: density gradient ultracentrifugation; F: fraction; FGB: fibrinogen beta chain

Supplementary Figure 6



Supplementary figure 6 – Comparison of EV isolation from fresh and frozen plasma by large-scale DGUC (Type 70.1 rotor) Average of protein content of fresh and frozen (data from Fig.3. C) samples (A) and representative Western blot images of frozen (data from Fig.3 C and D) (B), and fresh (C) EV isolates by large-scale DGUC. Data are expressed as mean $\pm$ standard error of mean. DGUC: density gradient ultracentrifugation; F: fraction; FGB: fibrinogen beta chain