Supplementary information

Salivary extracellular vesicles isolation methods impact the robustness of downstream biomarkers detection

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Fig. S1.

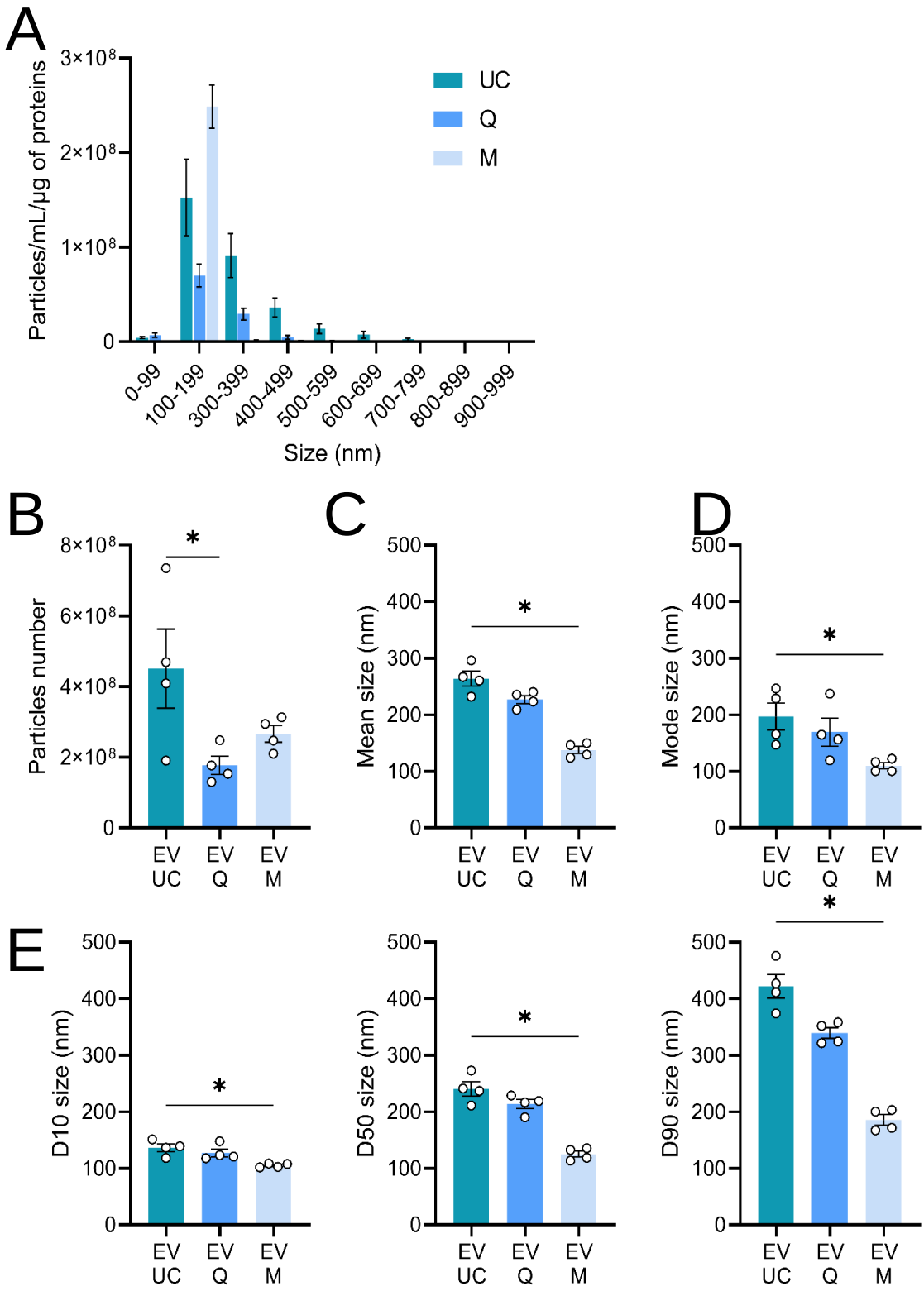


Fig. S1: Characterization of extracellular vesicles isolated from human saliva by their size and concentration with exclusion size under 100 nm for EV M. (A) Mean size distribution per range of 100 nm of extracellular vesicles (EV UC, Q and M) assessed by NanoTracking Analysis with exclusion size under 100 nm for EV M (n=4). (B) Mean size of EVs derived from saliva with exclusion size under 100 nm for EV M (n=4). (C) Mode size of EVs derived from saliva with exclusion size under 100 nm for EV M (n=4). (D) Particle size distribution D10, D50, and D90 (corresponding to the 10% smallest particles, 50% (median), and 10% largest particles within a sample respectively) with exclusion size under 100 nm for EV M (n=4). *: $p < 0.05$

Fig. S2.

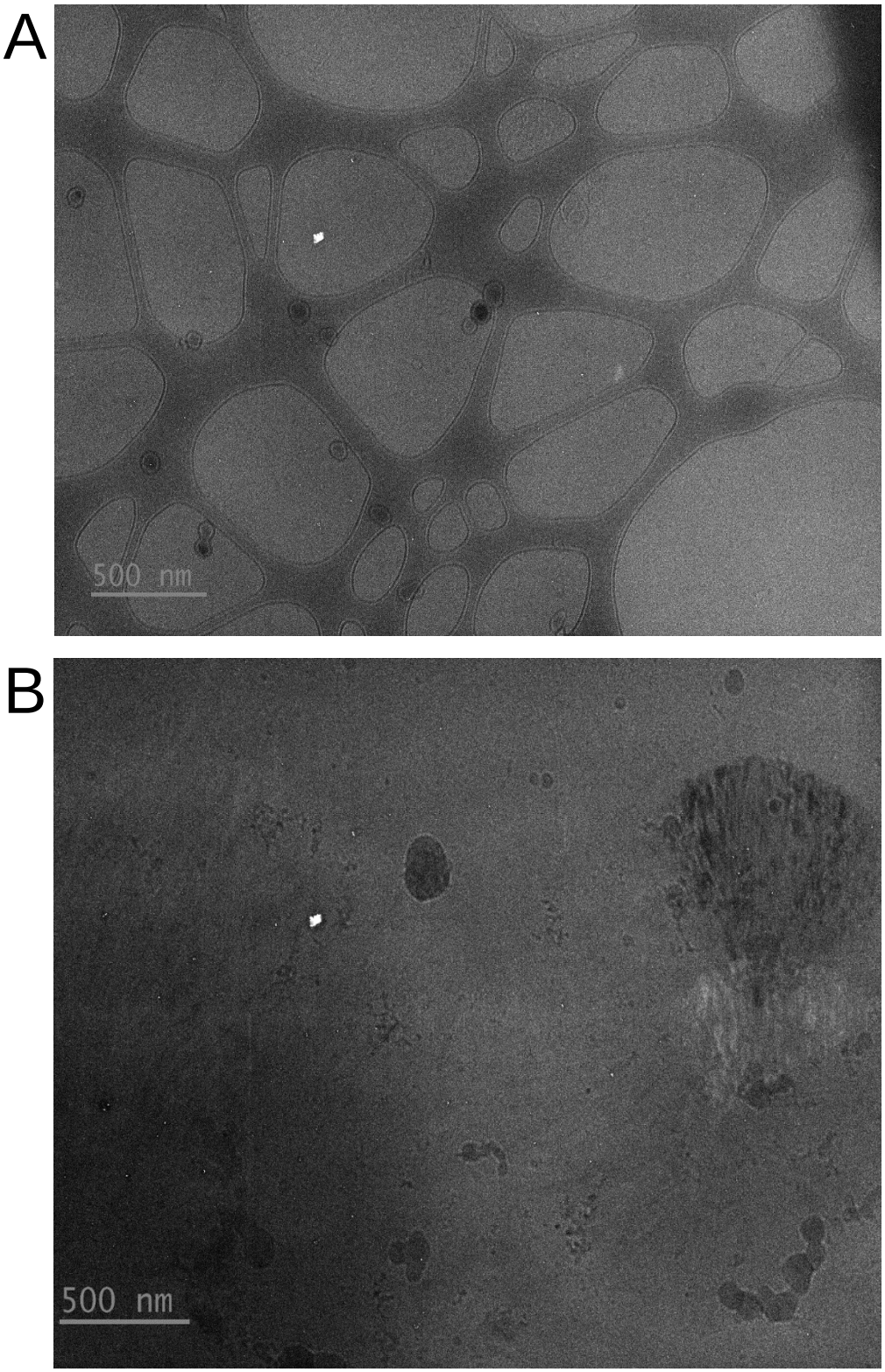


Fig. S2: Enlarged field vizualization of Evs by cryo-EM. (A) Representative picture of EVs isolated by ultracentrifugation (EV UC) by cryo-transmission electron microscopy. (B) Representative picture of EVs isolated by co-precipitation (EV Q) by cryo-transmission electron microscopy. Image bars represent 500 nm.

Fig. S3.

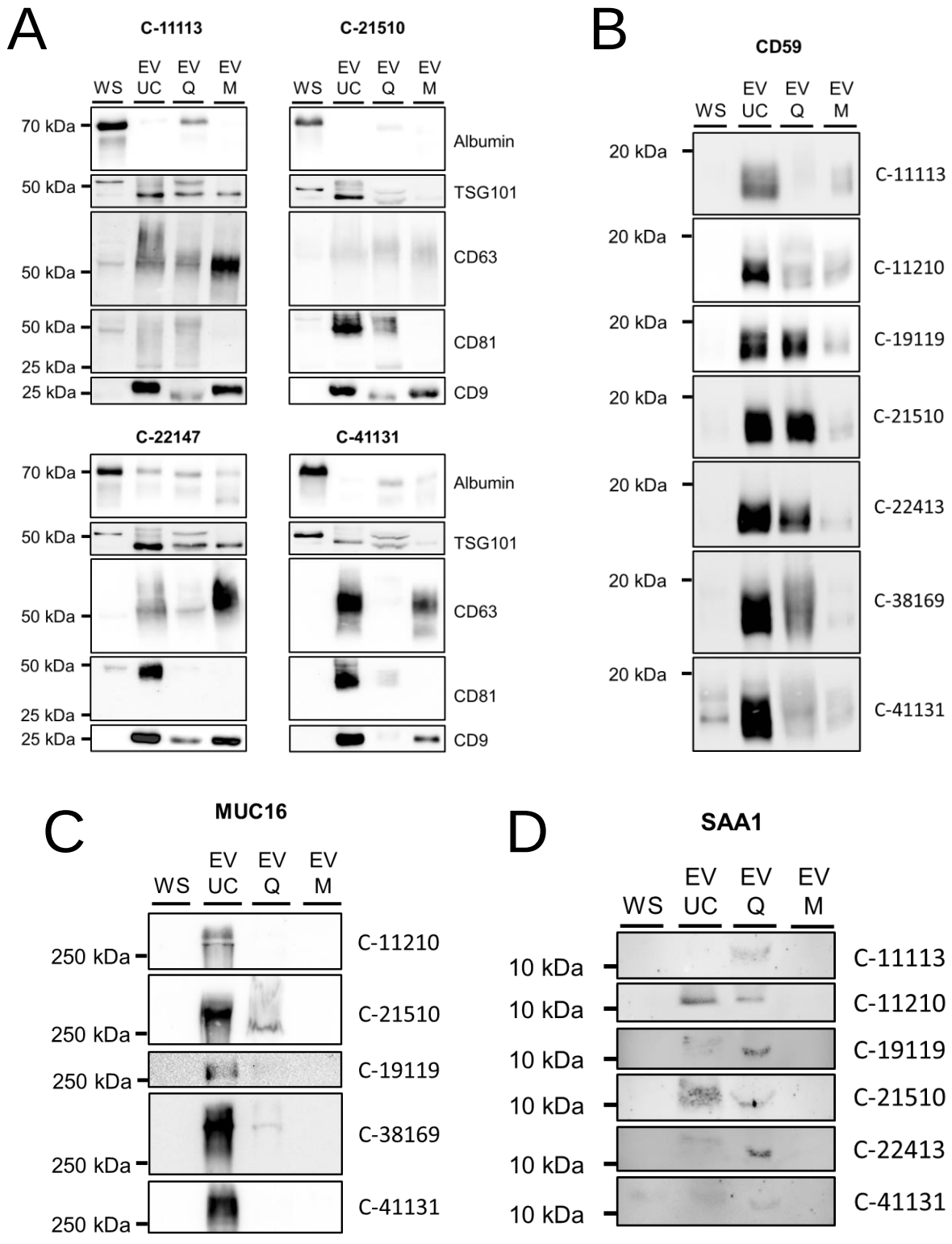
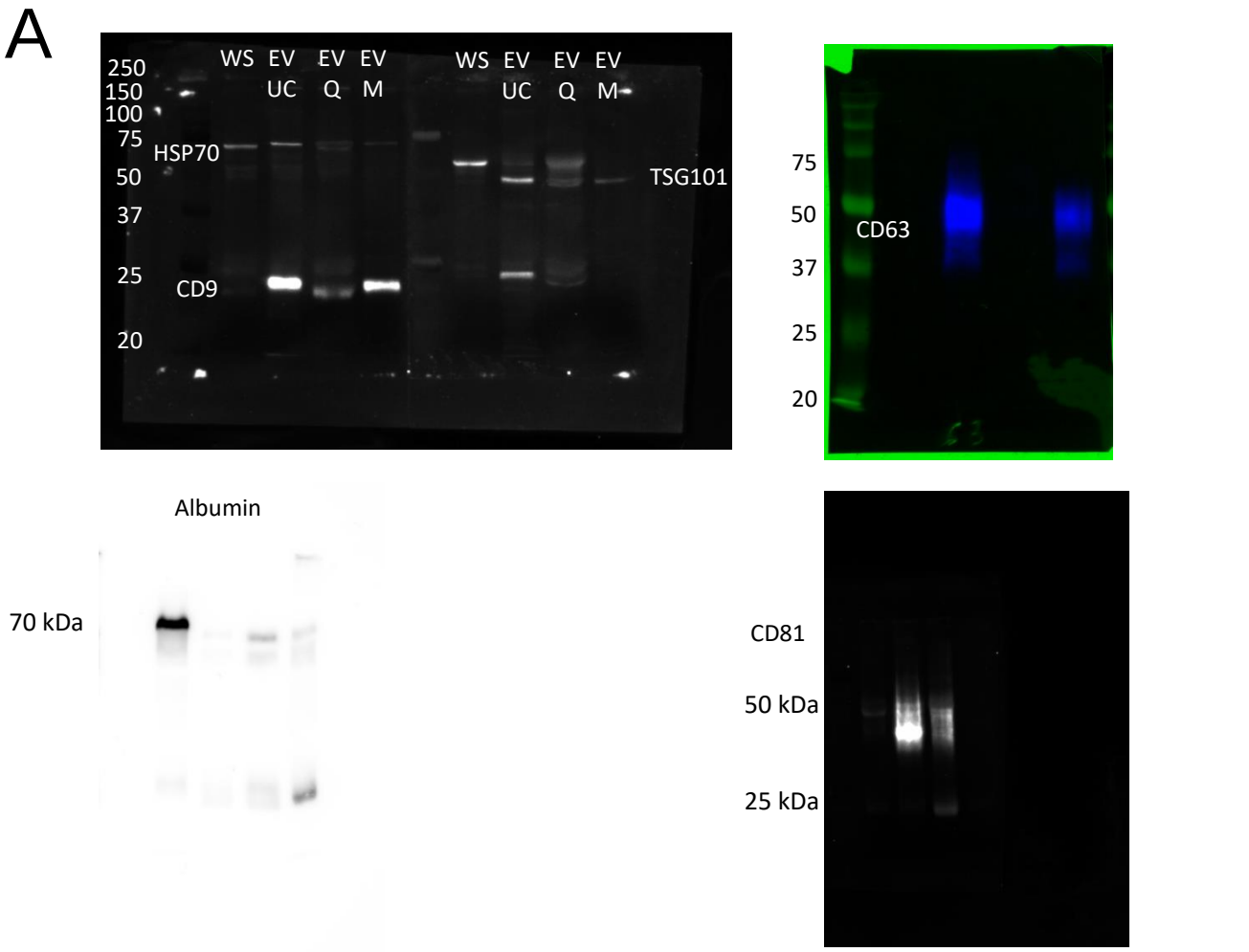


Fig. S3: Individual protein profiles for extracellular vesicles characterization by western blot. (A) Western blot analysis of (A) albumin, TSG 101 and tetraspanins (CD9, CD63, CD81) markers(n=4), (B) CD59 (n=7), (C) mucin-16 (n=5), (D) serum amyloid A1 (n=7) in WS, EV UC, EV Q and EV M protein extracts. Original blots are provided in Supplementary Fig. S8 (A) and S9 (B,C,D).

Fig. S4.



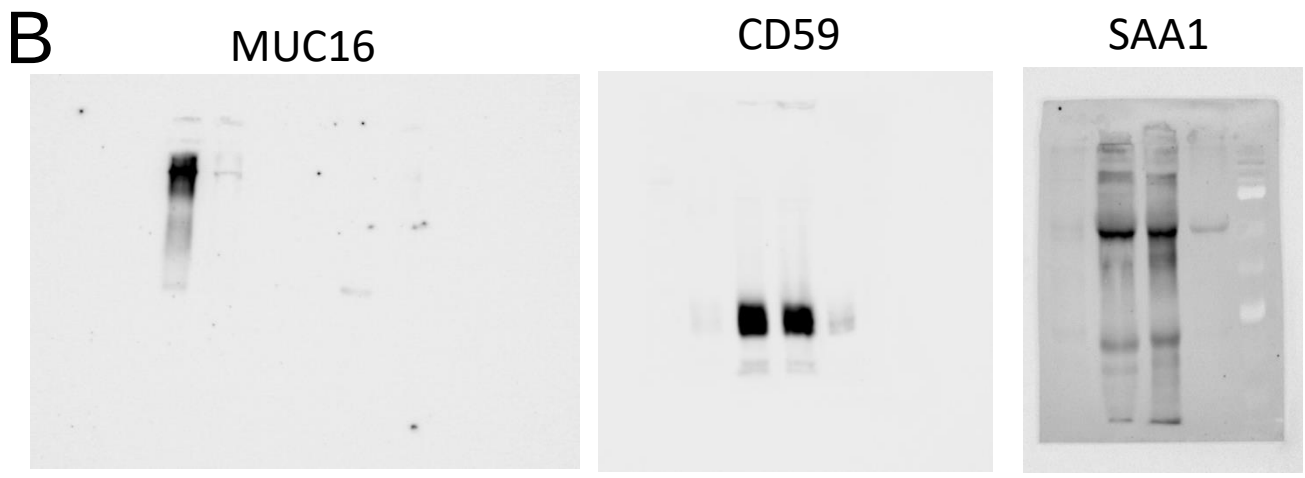


Fig. S4: Full-length and cutted membranes of all immunoblots related to Fig. 3. (A and B) Uncropped Western blots corresponding to Fig. 3C (A) and Fig. 3G (B). Molecular Weight are indicated on the left or on the right side of the membrane. Some membranes were cut prior to hybridization with antibodies.

Fig. S5.

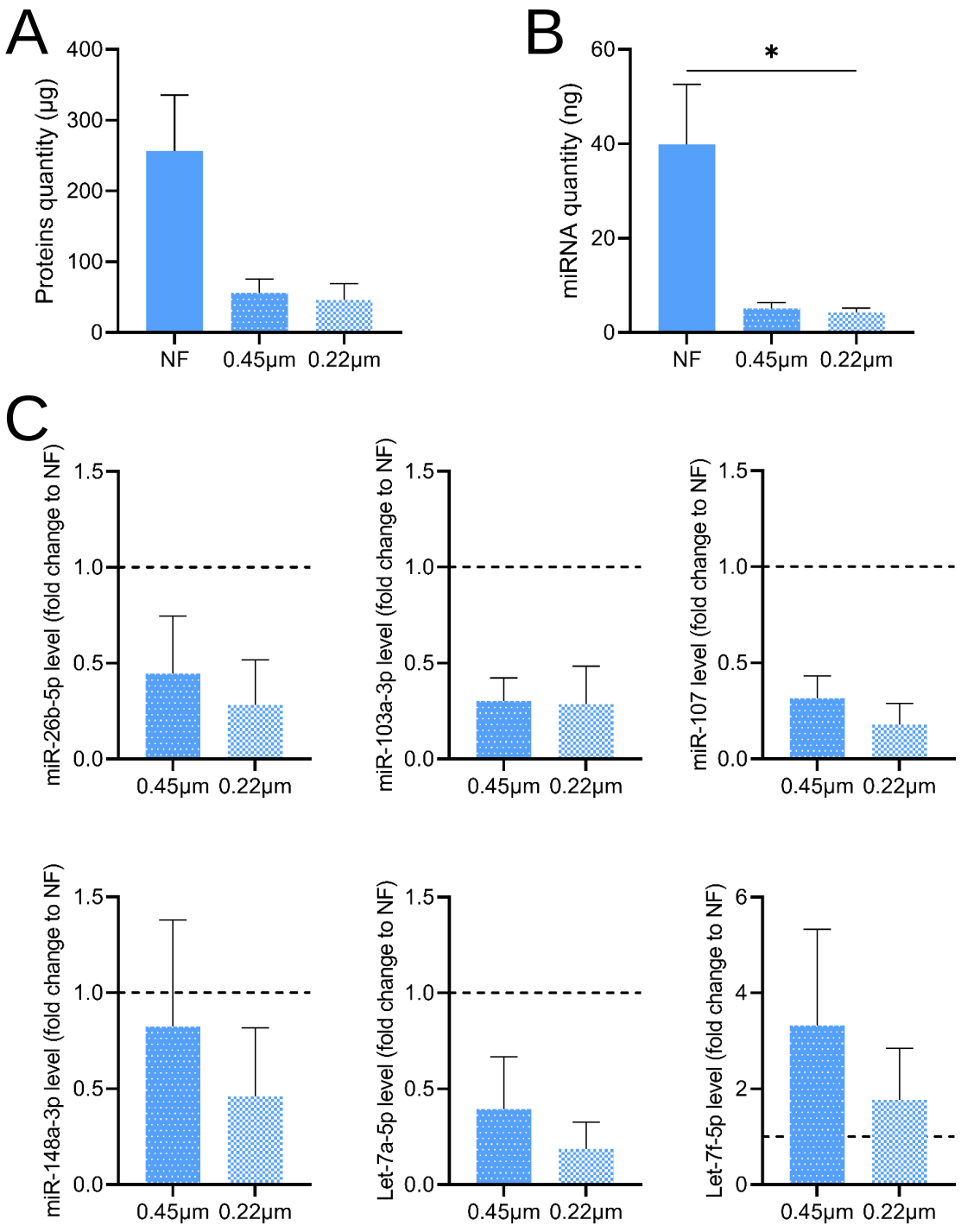


Fig. S5: Impact of filtration on saliva-derived extracellular vesicles isolated by co-precipitation. (A) Concentration of total proteins contained in EVs isolated from 1mL of human non-filtered saliva (NF) and 0.45µm or 0.22µm filtered saliva (n=3). (B) Quantity of total small RNA contained in EVs isolated from 1mL of human non-filtered saliva (NF) and 0.45µm or 0.22µm filtered saliva (n=4). (C) miRNA levels in EVs isolated from filtered 0.45µm or 0.22µm saliva relative to EVs isolated from non-filtered saliva (NF). Results are given as fold-change vs. control non-filtered saliva normalized at 1. (n=4). *: $p < 0.05$.

Fig. S6.

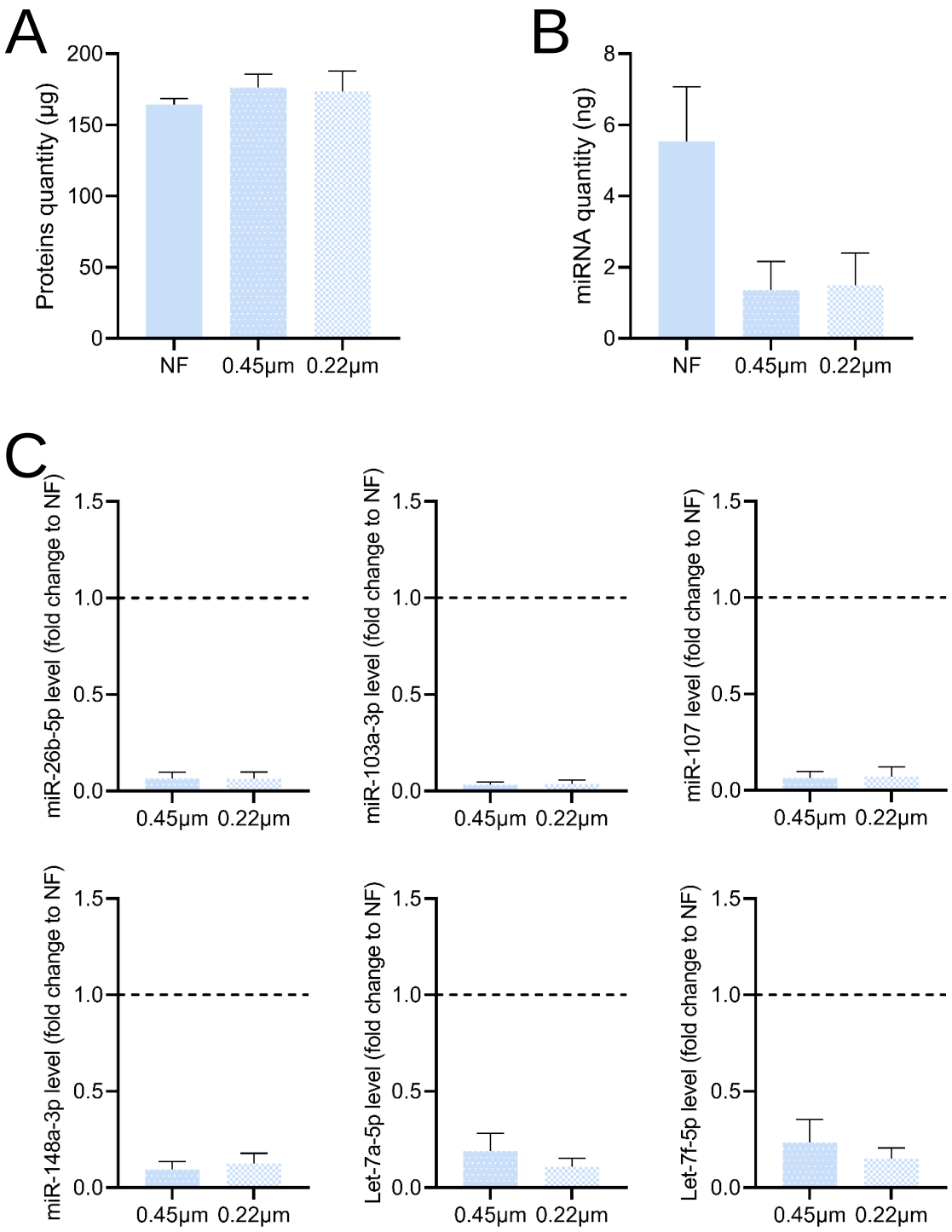


Fig. S6: Impact of filtration on saliva-derived extracellular vesicles isolated by immuno-affinity. (A) Concentration of total proteins contained in EVs isolated from 1mL of human non-filtrered saliva (NF) and 0.45µm or 0.22µm filtered saliva (n=3). (B) Quantity of total small RNA contained in EVs isolated from 1mL of human non-filtered saliva (NF) and 0.45µm or 0.22µm filtered saliva (n=4). (C) miRNA levels in EVs isolated from filtered 0.45µm or 0.22µm saliva relative to EVs isolated from non-filtered saliva (NF). Results are given as fold-change vs. control non-filtered saliva normalized at 1. (n=4). *: $p < 0.05$.

Fig. S7.

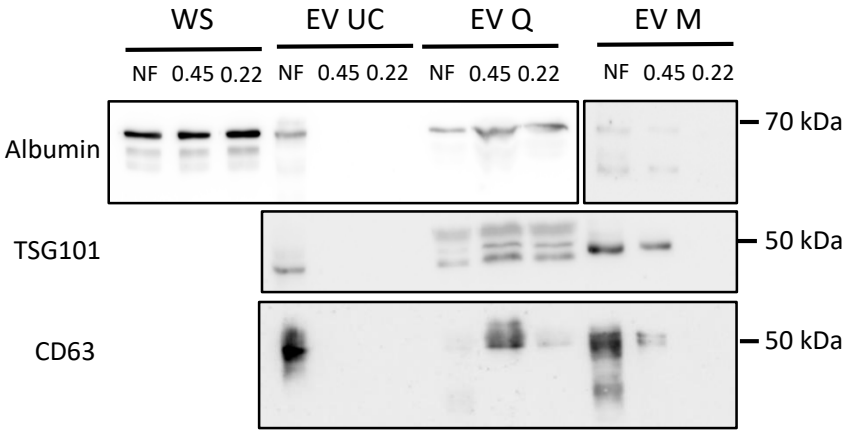


Fig. S7: Evaluation of protein profiles for extracellular vesicles characterization by western blot before and after filtration. Western blot analysis of albumin, TSG 101 and tetraspanin CD63 markers in WS, EV UC, EV Q and EV M protein extracts before (NF) and after filtration using 0.45 and 0.22 µm filters. Original blots are provided in Supplementary Fig. S10.

Fig. S8.

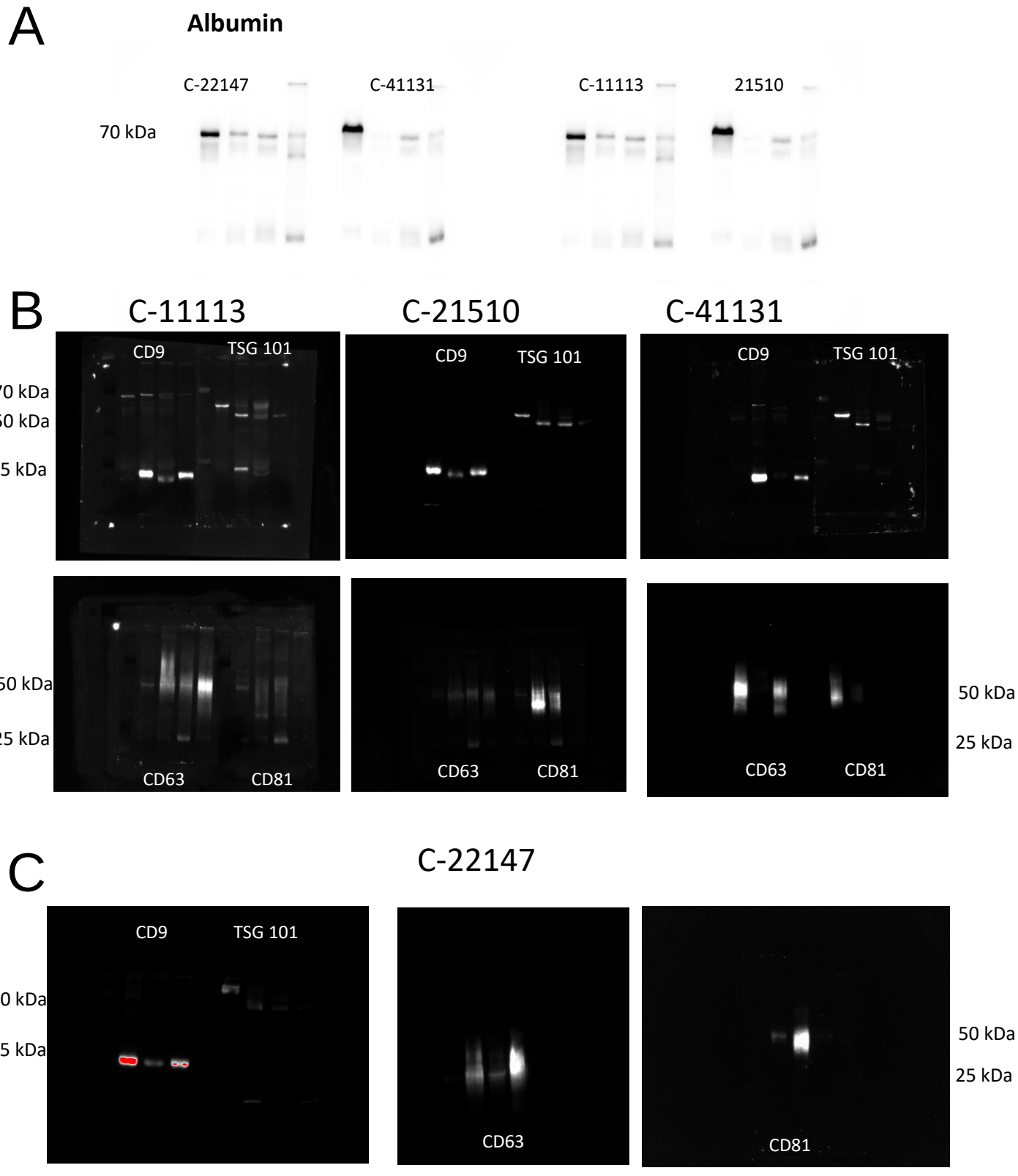


Fig. S8: Full-length and cutted membranes of all immunoblots related to Fig. S3. Uncropped Western blots corresponding to Fig. S3A. (A) Albumin immunoblots from C-11113, C-21510, C-22147 and C-41131 . (B) TSG101, CD9, CD63 and CD81 immunoblots from C-11113, C-21510 and C-41131 samples. (C) TSG101, CD9, CD63 and CD81 immunoblots from C-22147 sample. Molecular Weight are indicated on the left or on the right side of the membrane. Some membranes were cut prior to hybridization with antibodies.

Fig. S9.

Fig. S3B CD59

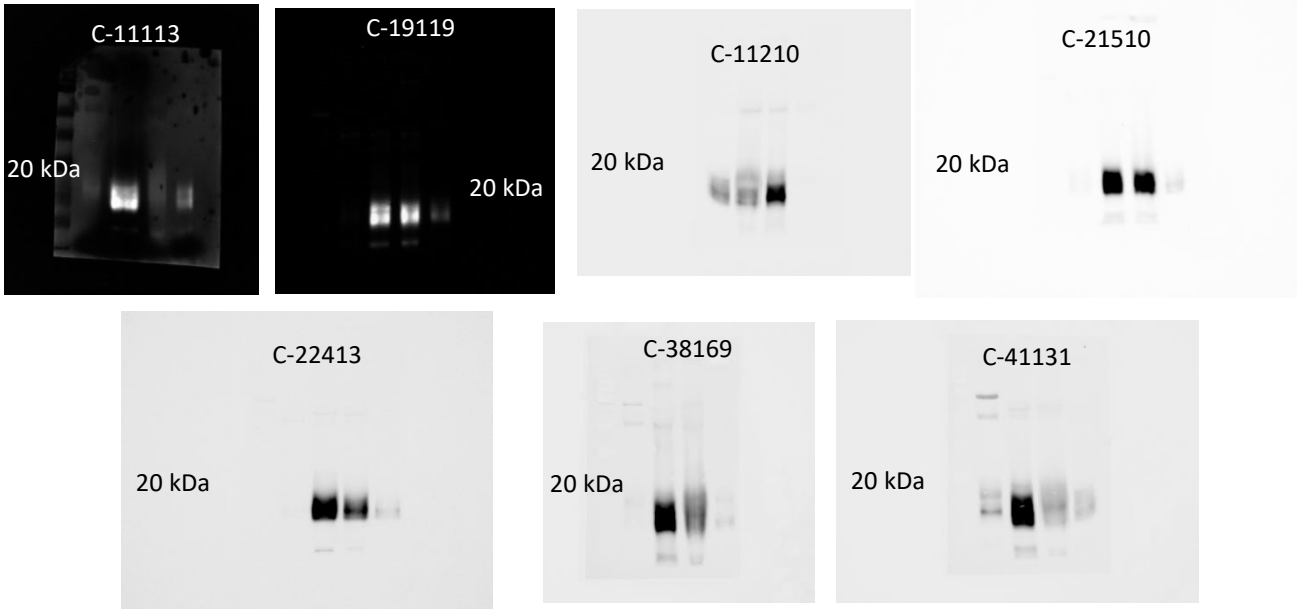


Fig. S3C MUC16

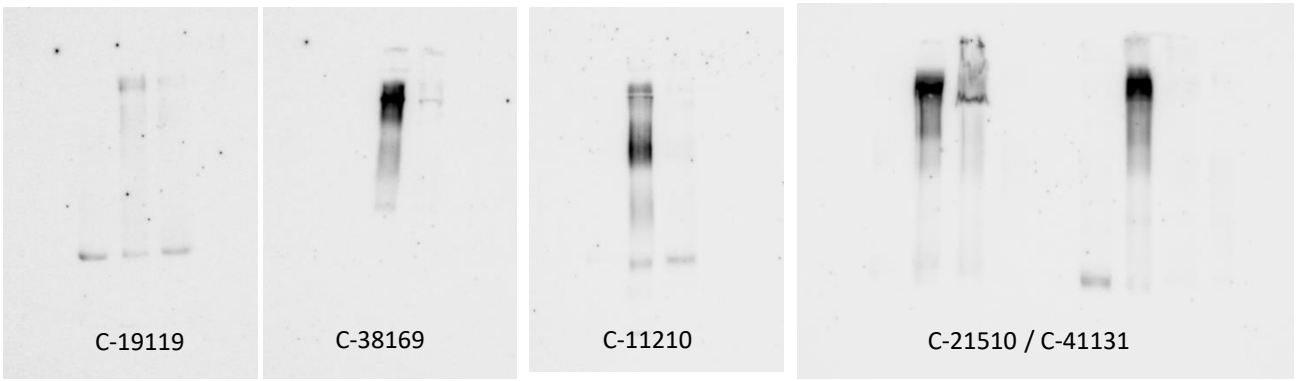


Fig. S3D SAA1

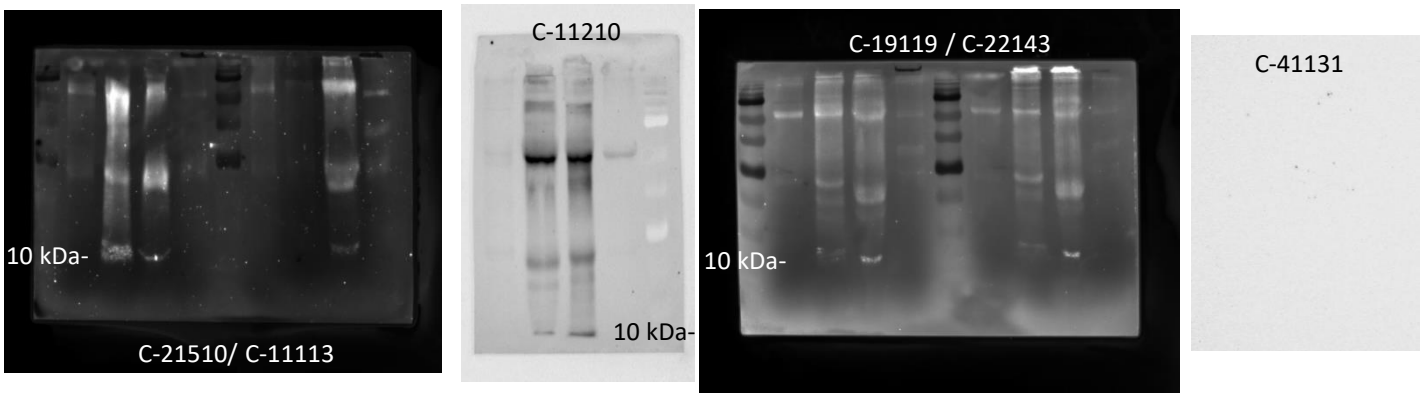


Fig. S9: Full-length and cutted membranes of all immunoblots related to Fig. S3. Uncropped Western blots corresponding to Fig. S3. B,C and D. Molecular Weight are indicated on the left or on the right side of the membrane. Some membranes were cut prior to hybridization with antibodies.

Fig. S10.

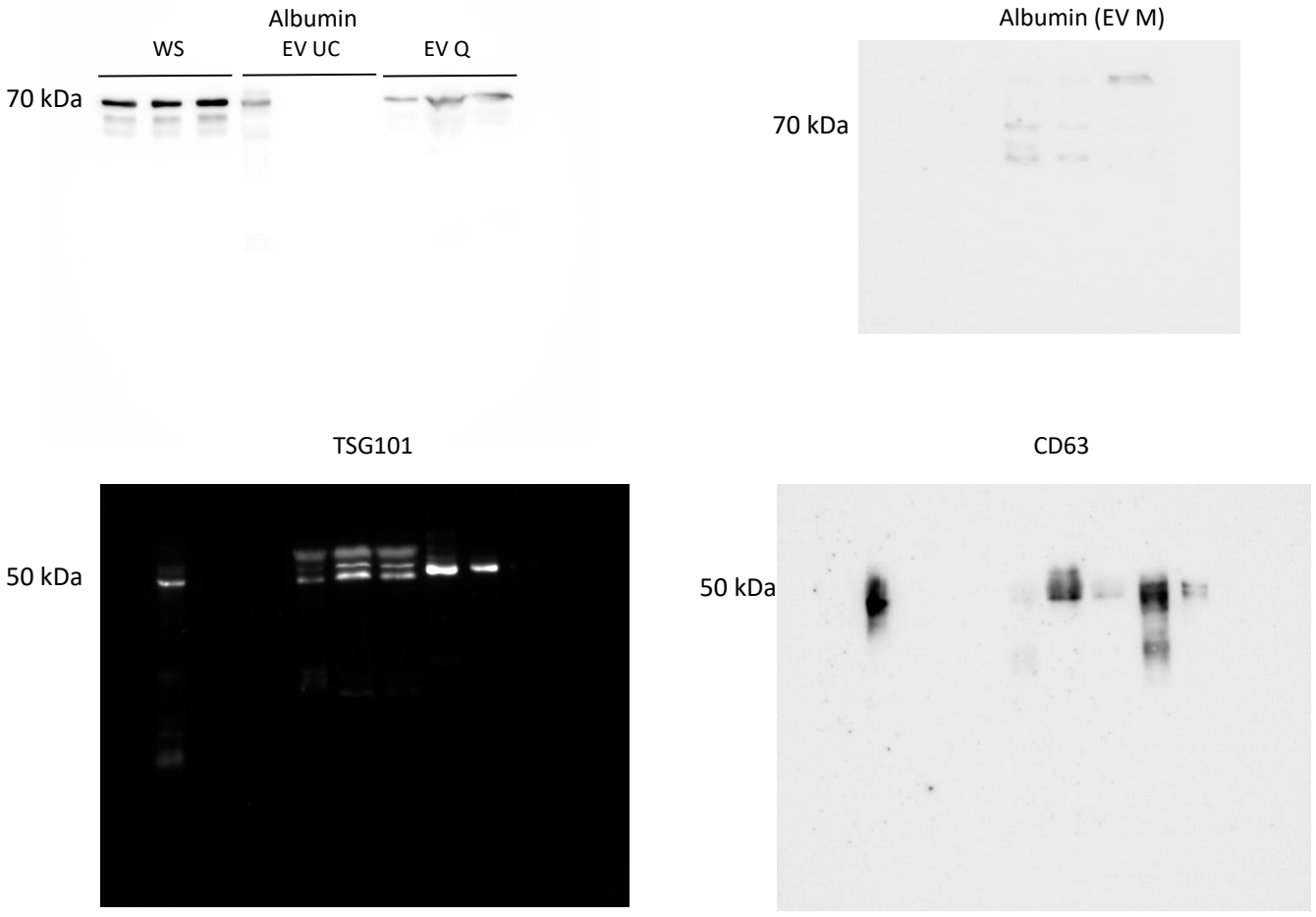


Fig. S10: Full-length and cutted membranes of all immunoblots related to Fig. S7. Uncropped Western blots corresponding to Fig. S7. Molecular Weight are indicated on the left side of the membrane. Some membranes were cut prior to hybridization with antibodies.