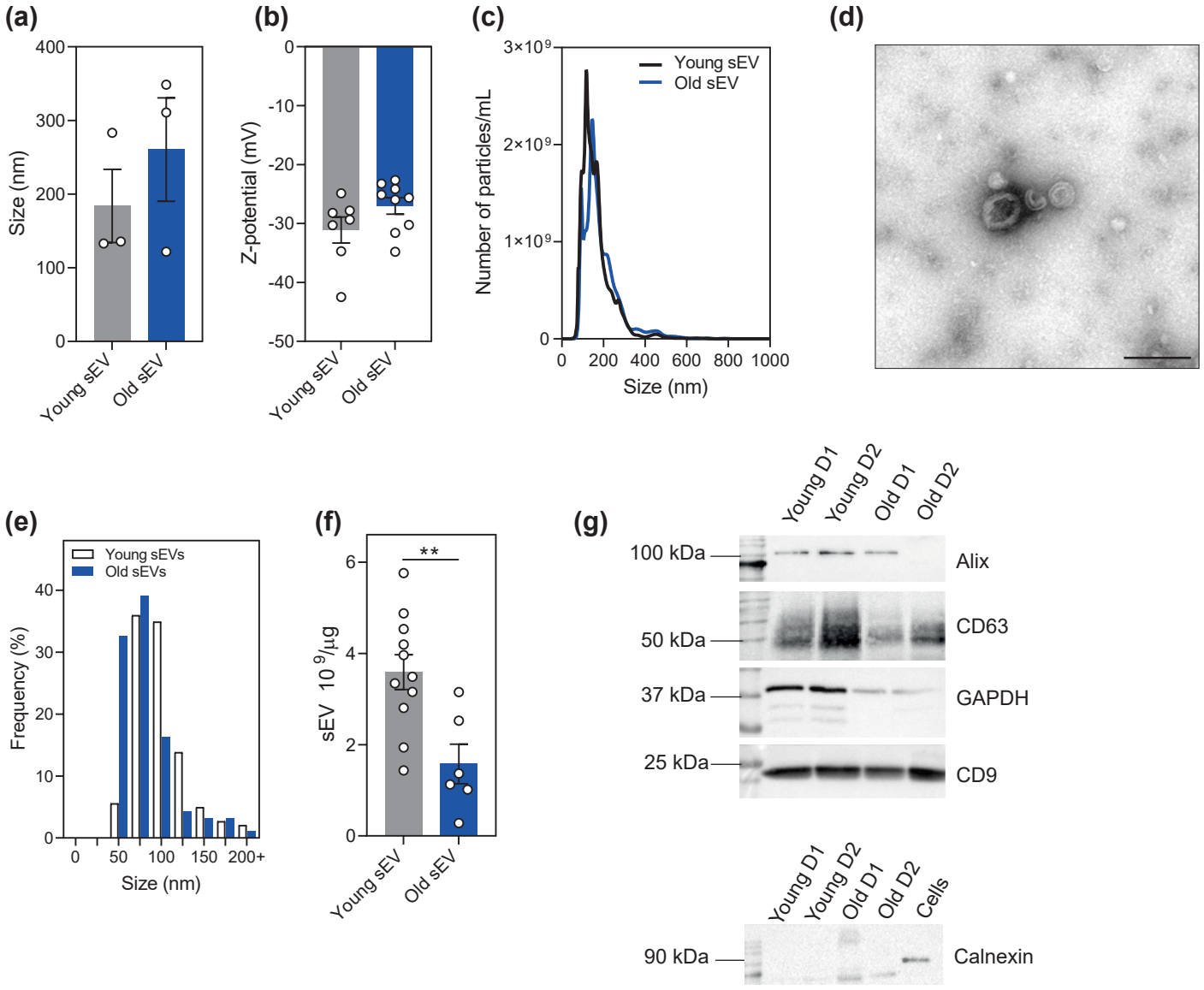

Figure S1 – Characterization of sEVs from young and from aged donors according to MISEV guidelines. The young and old sEVs were isolated from human UCB and blood from adult donors, respectively. (a) Size analysis and (b) zeta potential of young and old sEVs determined by DLS. Results are expressed as mean ± SEM (n = 3 independent experiments with 1-3 measurements per n). (c) NTA analysis of young and old sEVs (each curve is the average of n = 3 independent experiments). (d) Representative TEM image of old sEVs. Scale bar is 200 nm. (e) Distribution frequency of the diameter of the young and old sEVs (8-10 images were analysed for each condition). (f) Total protein quantification of young and old sEVs through microBCA assay. Data are expressed as mean ± SEM (n = 6-11 independent sEVs isolations). Statistical analysis was performed using an unpaired t-test, ** means p value < 0.01. (g) Western blot of young and old sEVs for evaluation of the specific EVs markers or contaminants. The D1 and D2 identify different donors of young and old sEVs, and ECs were used as a control. Old sEVs D1 = male 64 years old, Old sEVs D2 = female 64 years old.

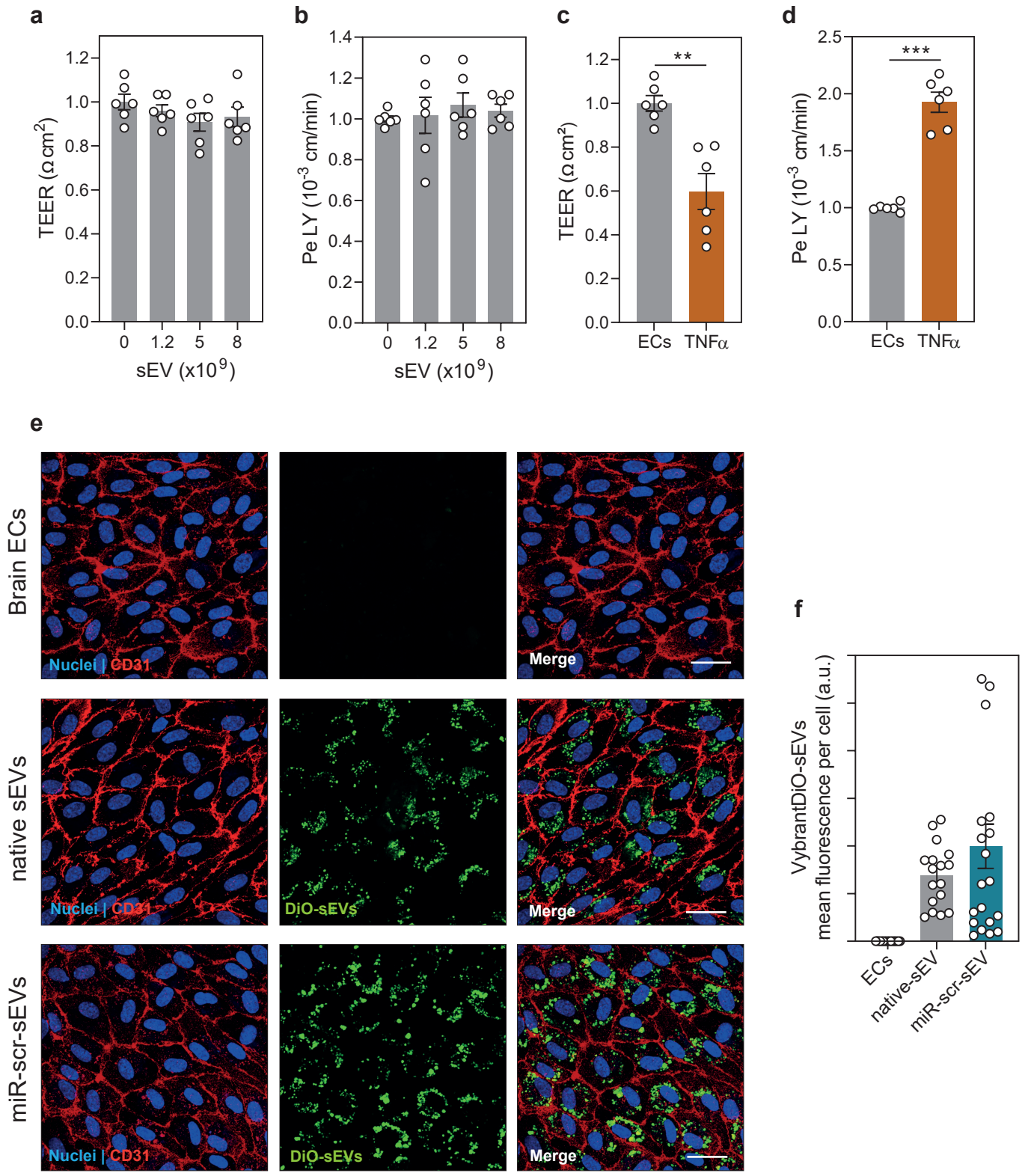

Figure S2 – Evaluation of native sEV effect in BBB properties and sEV cellular internalization. (a) The TEER of ECs monolayer and (b) paracellular permeability to LY was evaluated after the incubation for 48 h of different concentrations of young native sEVs. Results are expressed as mean ± SEM (n = 2 independent experiments with 3 technical replicates). (c) TEER evaluation and (d) paracellular permeability to LY of the BBB model in in the presence of the TNFα for evaluation of functional response of the BBB model. Results are expressed as mean ± SEM (n = 2 independent experiments with 3 technical replicates). Unpaired t test was performed, ** means p value < 0.01, *** means p < 0.001. (e) Representative confocal images of the brain ECs after the incubation with 1010 VybrantDiO-sEVs/mL. The labeled native sEVs or labeled miR-scr-enriched-EVs were incubated for 48 h with the BBB model. The ECs without sEVs incubation were used as a control. Scale bar is 30 µm. (f) Quantification of the fluorescence intensity of the Vybrant-DiO-sEVs divided by the number of nuclei in each image. Results are expressed as mean ± SEM (n = 4 independent experiments with 2-3 technical replicates).

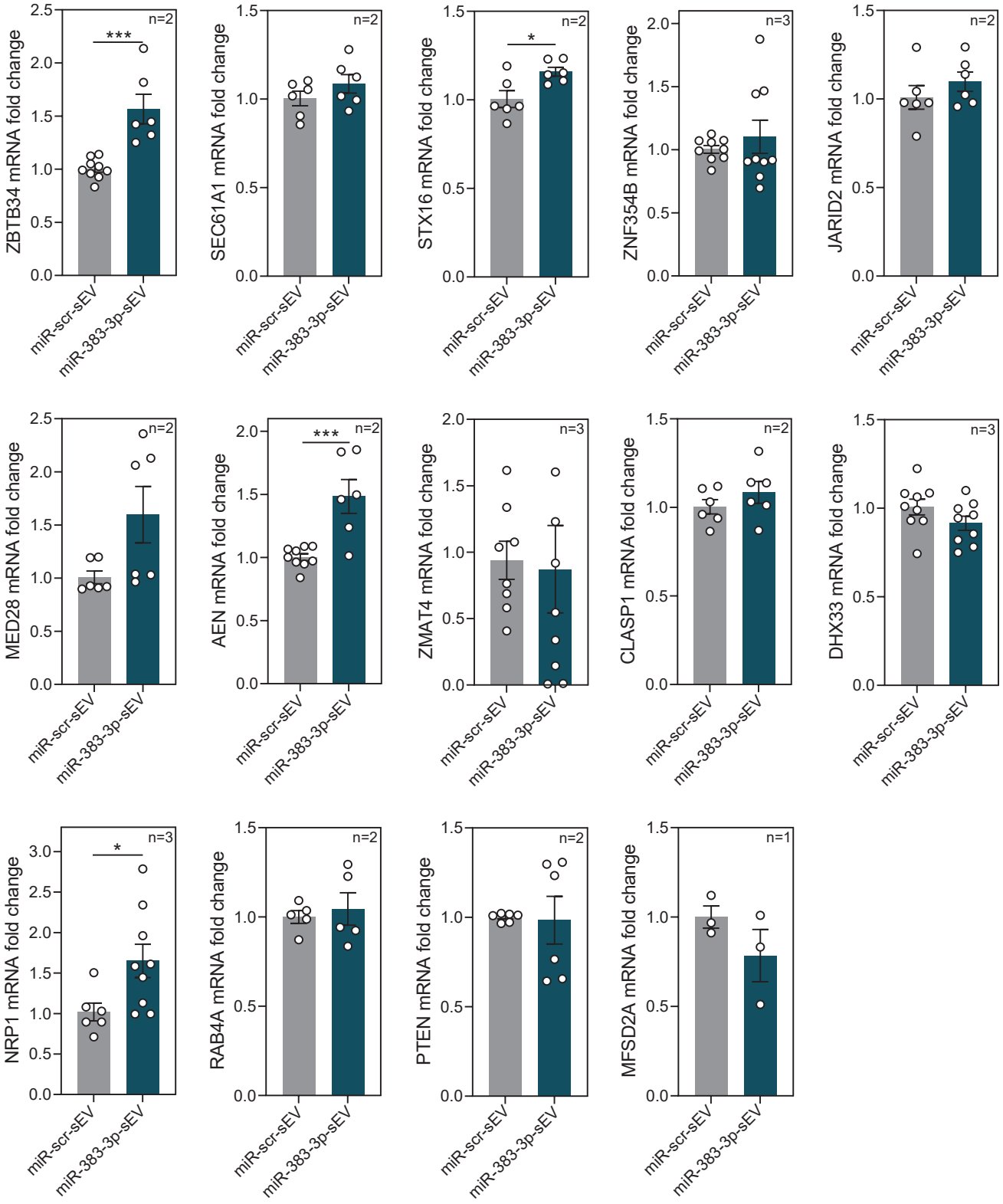

Figure S3 – mRNA expression in brain ECs after transfection with the miR-383-3p-enriched-sEVs. mRNA expression in brain ECs cultured in the static BBB model and transfected with miR-383-3p-enriched-sEVs or miR-scr-enriched-sEVs (10^{10} sEVs) for 48h. The results are expressed as mean ± SEM (average of n = 1-3 independent experiments with 3 technical replicates). Unpaired t-test statistical analysis was performed, * $p < 0.05$, *** $p < 0.001$.