

Supplementary Material

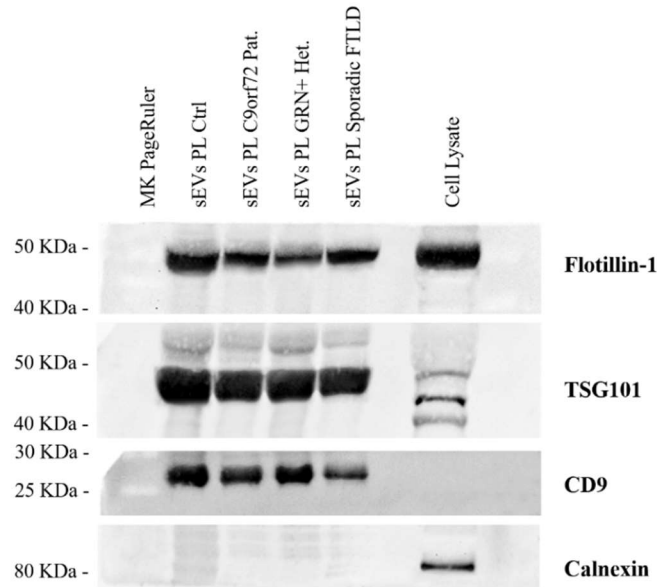


Figure S1. Plasma sEV characterization. Isolated plasma sEVs were CD9+ (a tetraspanin), TSG101+ and Flotillin-1+ (cytosolic proteins recovered in EVs) and Calnexin negative (endoplasmic reticulum residential protein, absent in EVs). Western Blot analysis on 4 representative samples (Ctrl, C9orf72 Pat., GRN+ Het., Sporadic FTLD) showed a decreasing trend in TSG101, Flotillin-1, and CD9 expression in sEVs from *C9orf72* pathological expansion carriers, GRN mutation carriers, and sporadic FTLD patients compared to controls.

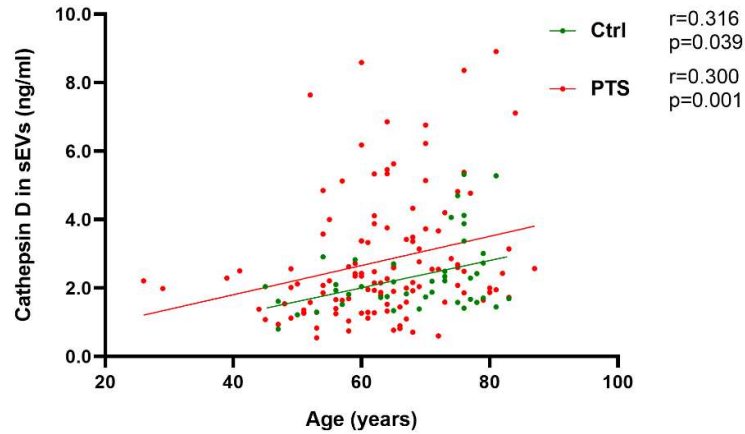
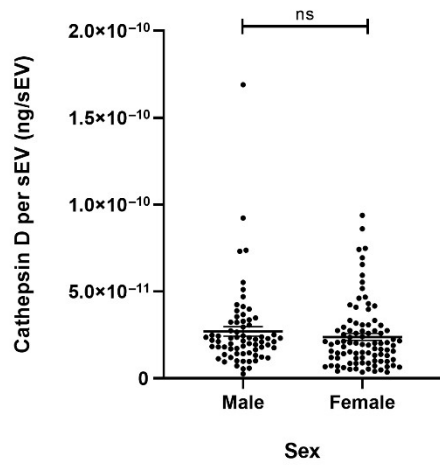
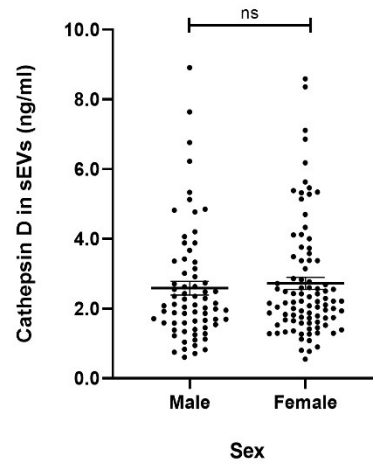


Figure S2. Correlation of total cathepsin D in sEVs and age. Correlation of total cathepsin D content in sEVs (ng/ml, not normalized values) with age in controls (Ctrl, in green) and patients (PTS, in red). Age was positively correlated with total cathepsin D in sEVs both in PTS (Spearman $r=0.316$, $p=0.039$) and in Ctrl (Spearman $r=0.300$, $p=0.001$). Spearman correlation r and p -values are shown in the panel.

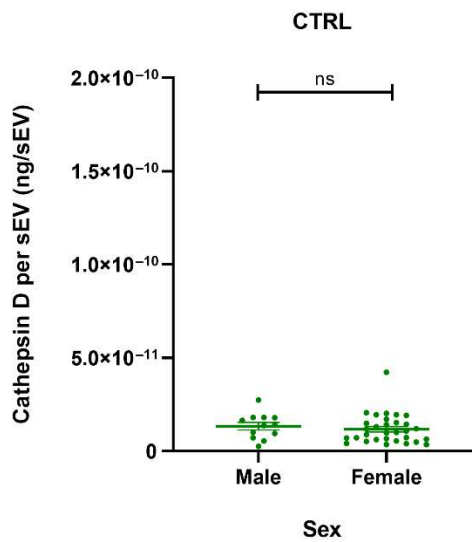


(a)

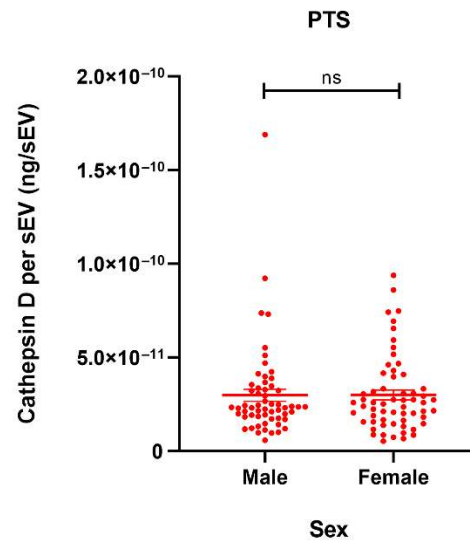


(b)

Figure S3. No sex-related differences were observed in sEV cathepsin D levels. (a) Cathepsin D concentration per sEV (ng/sEV) and (b) total cathepsin D in sEVs (ng/ml) differences between male and females ($p=0.139$ and $p=0.470$ respectively, Mann-Whitney test).



(a)



(b)

Figure S4. No sex-related differences were observed in Cathepsin D concentration per sEV (ng/sEV) in (a) CTRL and (b) PTS between males and females ($p=0.543$, Unpaired t-test and $p=0.827$, Mann-Whitney test, respectively).

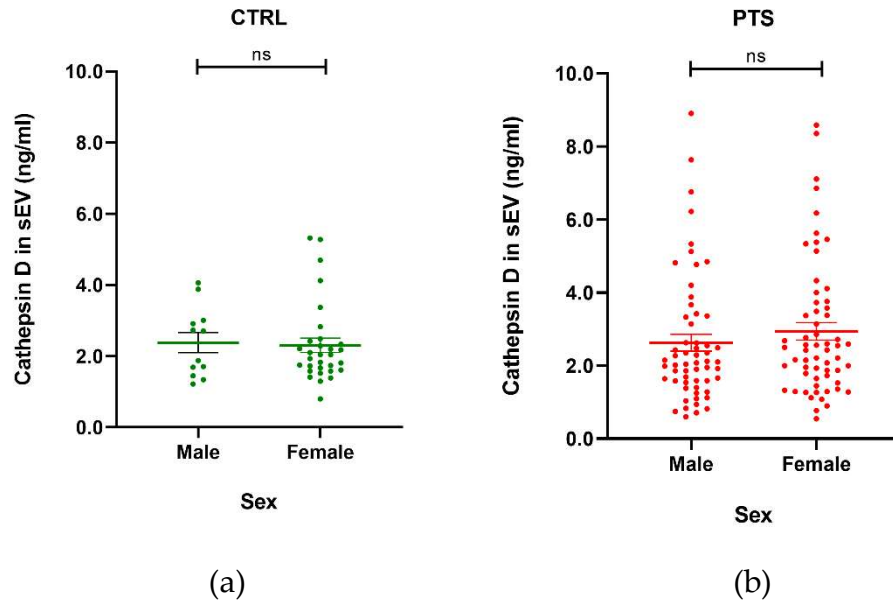


Figure S5. No sex-related differences were observed in total cathepsin D in sEVs (ng/ml) in (a) CTRL and (b) PTS between males and females ($p=0.815$ and $p=0.208$ respectively, Mann-Whitney test).

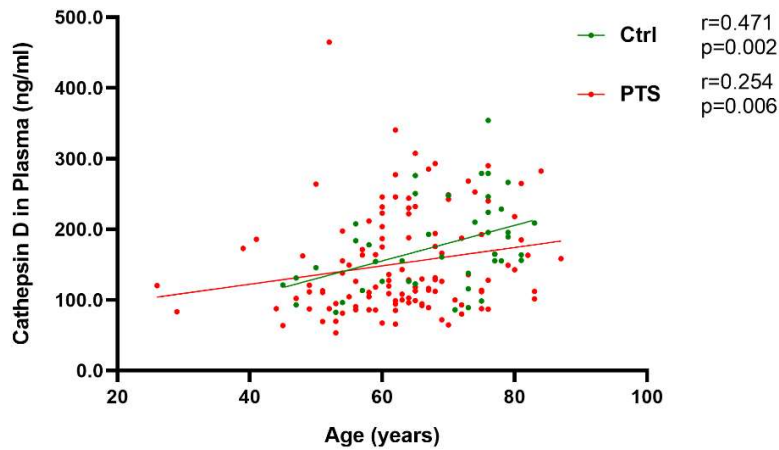


Figure S6. Correlation of cathepsin D concentration in plasma and age. Correlation of cathepsin D concentration in plasma (ng/ml) with age in controls (Ctrl, in green) and patients (PTS, in red). Age was positively correlated with plasma cathepsin D both in PTS (Spearman $r=0.254$, $p=0.006$) and in Ctrl (Spearman $r=0.471$, $p=0.002$). Spearman correlation r and p -values are shown in the panel.

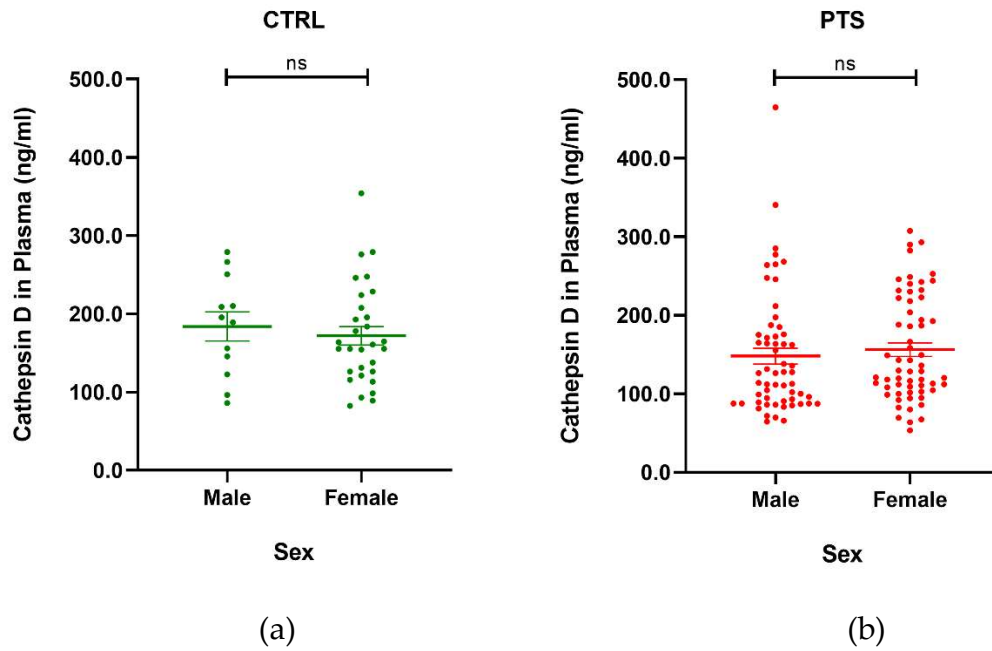


Figure S7. No sex-related differences were observed in plasma cathepsin D (ng/ml) in (a) Ctrl and (b) PTS between males and females ($p=0.587$ and $p=0.238$ respectively, Mann-Whitney test).

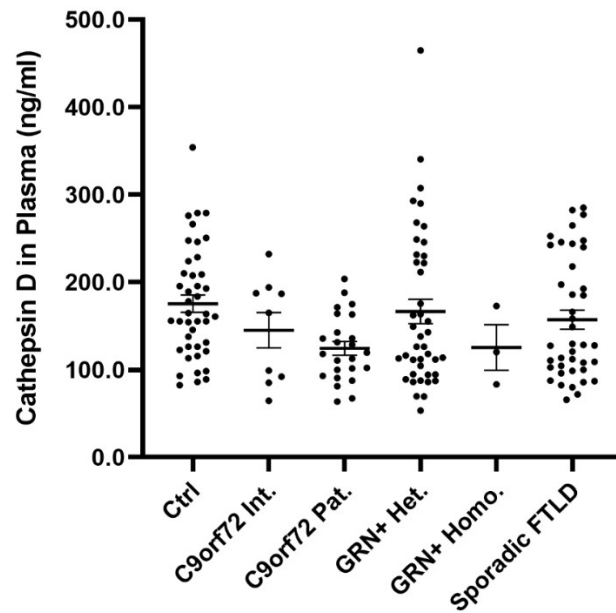


Figure S8. Cathepsin D concentration in plasma (ng/ml) in Ctrl, C9orf72 Int., C9orf72 Pat., GRN+ Het., GRN+ Homo., and Sporadic FTL. Mean \pm SEM. Dot plot represents raw data while the global p -value ($p=0.055$) was obtained by generalized linear model with inverse gaussian distribution adjusted for age and sex.

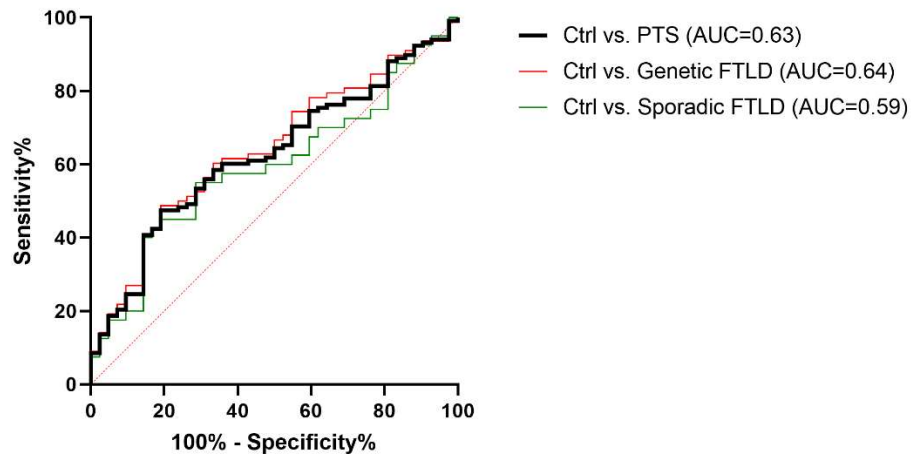


Figure S9. ROC curves for plasma cathepsin D. Plasma cathepsin D concentration was used to evaluate the discrimination of PTS from Ctrl. AUC Ctrl vs PTS 0.63 (black); AUC Ctrl vs Genetic FTLD 0.64 (red); AUC Ctrl vs Sporadic FTLD 0.59 (green). AUC comparison with DeLong test, $p=0.565$.