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Analysis of EV Marker Levels in Blood Samples from Patients with Neurodegenerative Diseases Using EV ELISA

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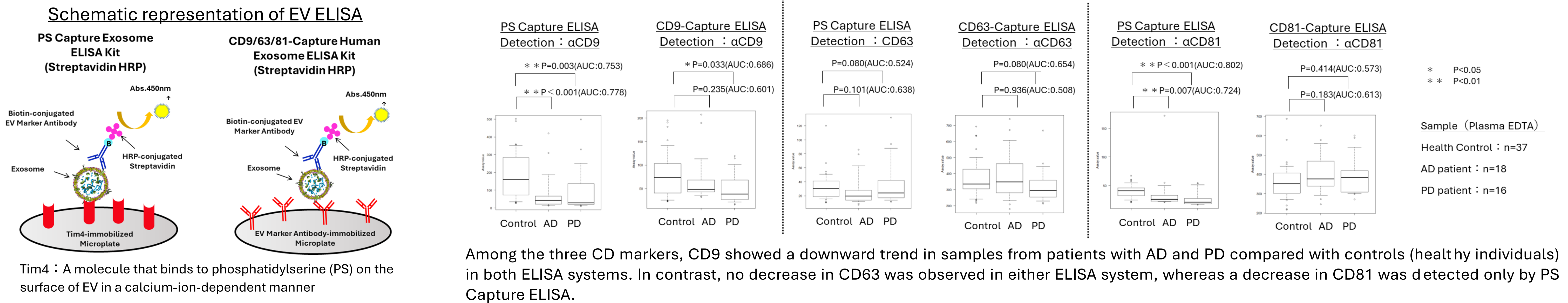
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1.Abstract

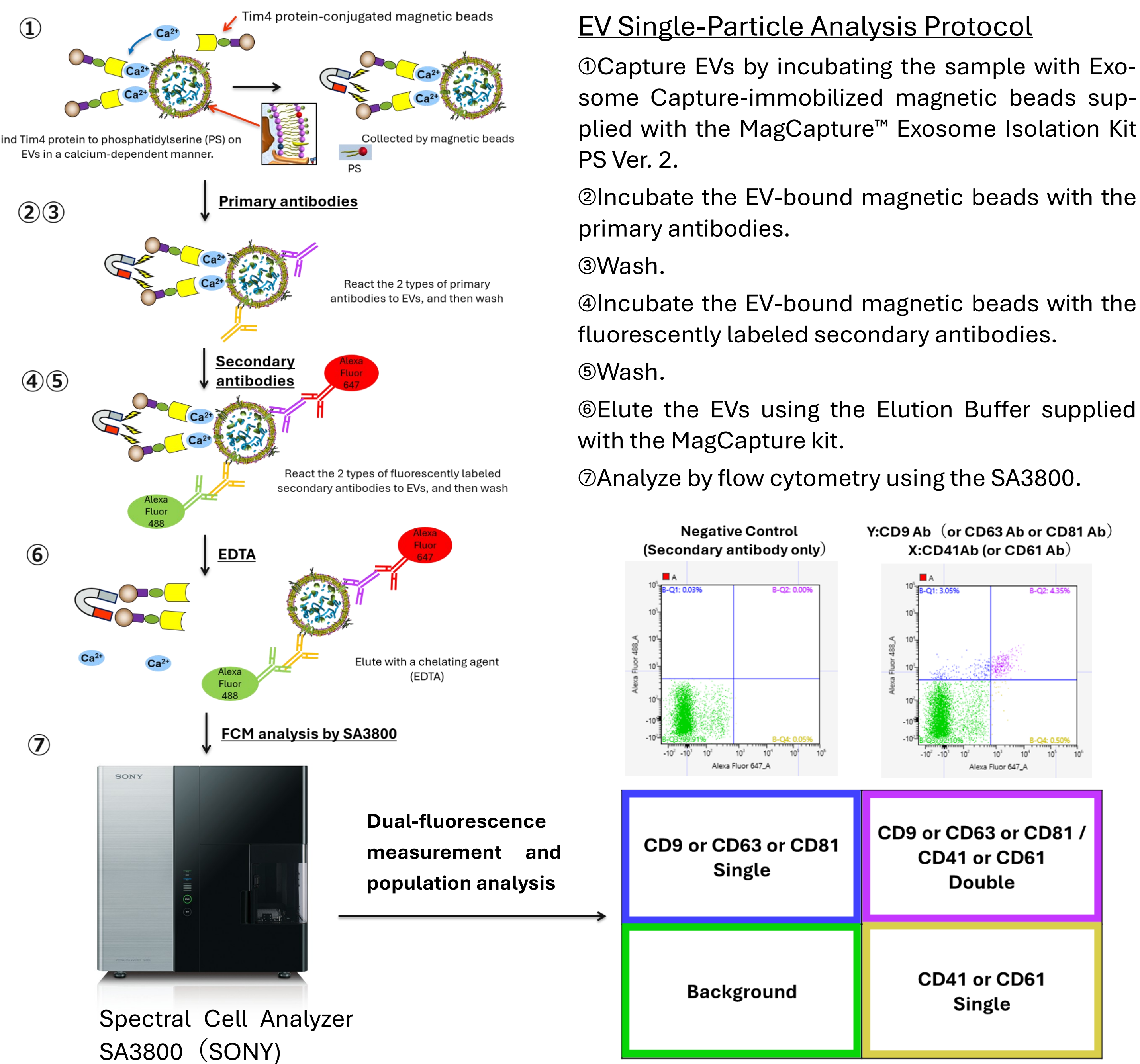
Extracellular vesicles (EVs) are membrane-bound vesicles carrying cell-derived nucleic acids, proteins, lipids, and their presence in body fluids suggests potential as biomarkers for low-invasive early diagnosis of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). In this study, to monitor changes in blood EV levels in neurodegenerative diseases, we measured the levels of EV markers CD9, CD63, and CD81 in blood samples from healthy individuals and patients with neurodegenerative diseases using PS Capture ELISA, which captures EVs via Tim4 that binds phosphatidylserine (PS) on the EV surface in a calcium-dependent manner, and antibody sandwich ELISA, which detects EVs using antibodies against EV markers. The results confirmed that CD9 was significantly reduced in plasma samples from AD and PD patients in both ELISA systems. Since a decrease in the amount of CD9-positive EVs was suggested in the blood of patients with neurodegenerative diseases, we investigated the relationship with platelet-derived EVs as part of the characterization of CD9-positive EVs. Single-particle analysis of EVs using FCM revealed that approximately 90% of platelet marker CD41-positive EVs and CD61-positive EVs co-express CD9. Furthermore, both ELISA systems showed that CD41 and CD61 levels in EVs in the plasma of patients with neurodegenerative diseases tended to be lower than in healthy individuals. These findings suggest a potential reduction in platelet-derived EVs in the blood of patients with neurodegenerative diseases.

2.EV Marker Measurement by EV ELISA

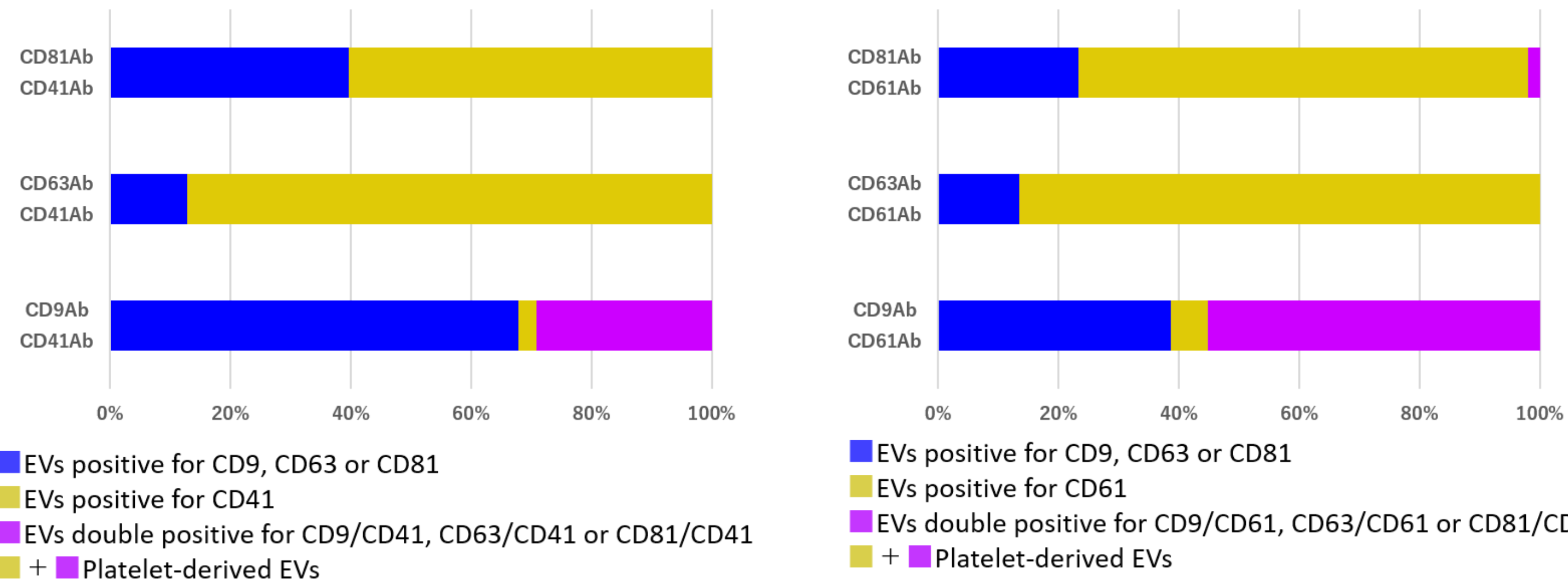
Plasma samples from healthy individuals and patients with neurodegenerative diseases (AD and PD) were analyzed to quantify CD9, CD63, and CD81 using a Tim4-based PS-affinity ELISA (PS Capture ELISA) and antibody sandwich ELISAs (CD9/CD63/CD81 Capture ELISA). Purified EVs derived from COLO201 cells and positive for CD9, CD63, and CD81 were used as ELISA standards to calculate assay values.



3.Single-particle analysis of PS-positive EVs

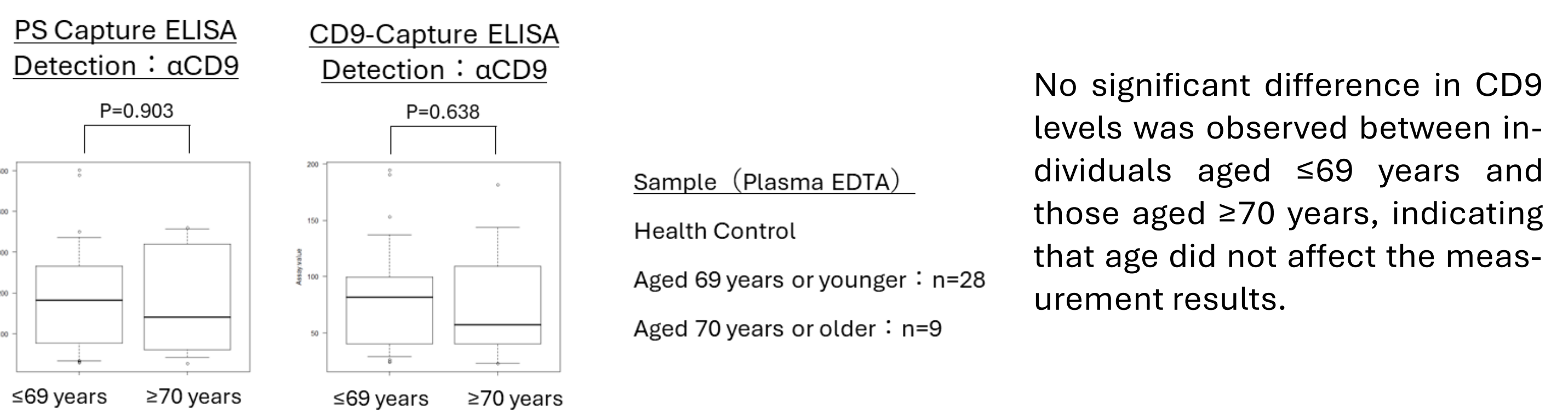


Individual EDTA plasma samples from healthy donors were used to assess the co-expression of the EV markers CD9, CD63, and CD81 and the platelet markers CD41 and CD61 on PS-positive EVs by single-particle flow cytometric analysis (FCM). EVs expressing only the EV markers (CD9, CD63, or CD81) are shown in blue, those expressing only the platelet markers (CD41 or CD61) in yellow, and those co-expressing both EV and platelet markers in purple.



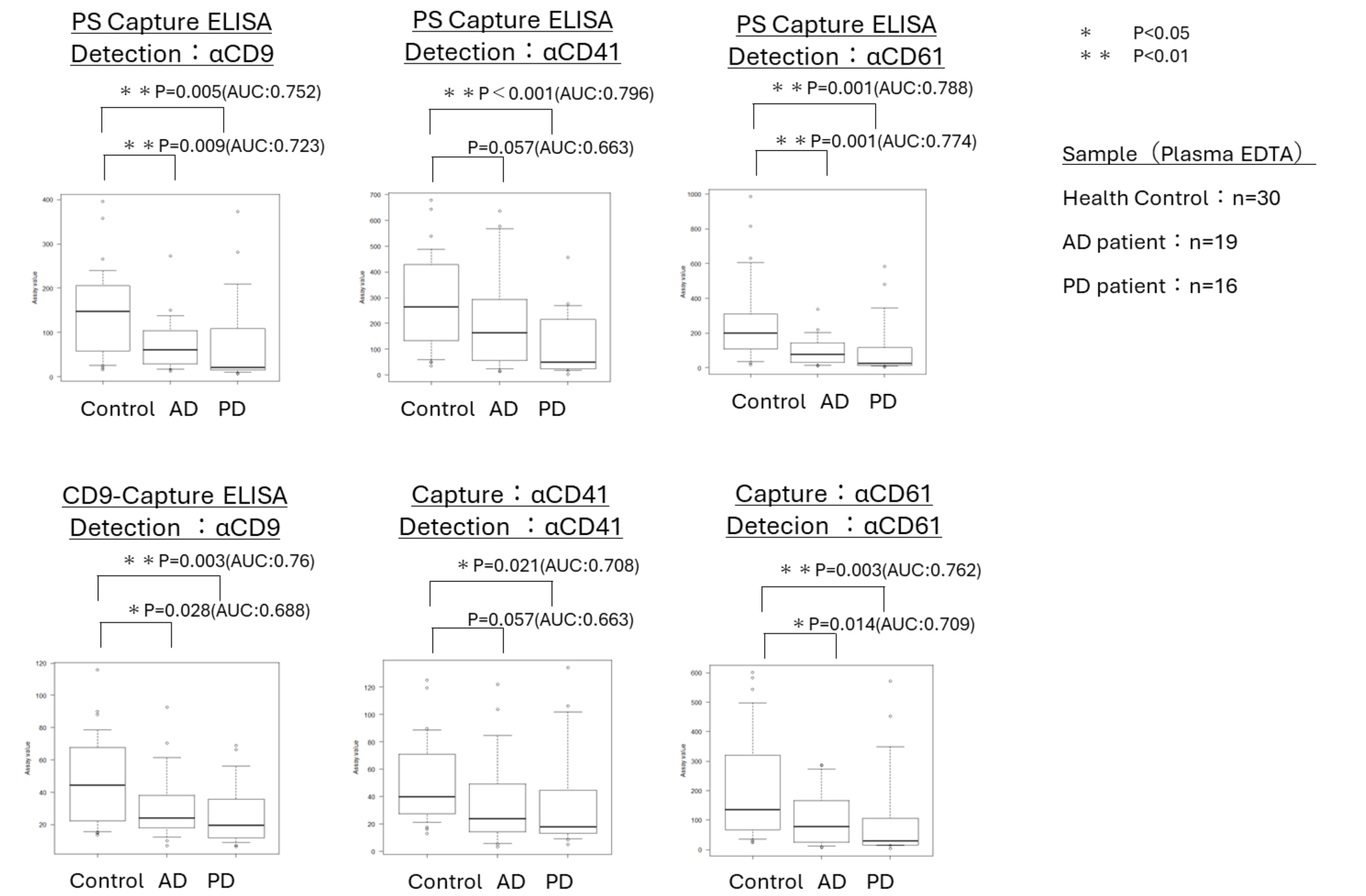
Of the CD41- or CD61-positive platelet-derived EVs (yellow + purple), approximately 90% co-expressed CD9 (purple), whereas only a few percent or less co-expressed CD63 or CD81. These findings confirmed that most platelet-derived PS-positive EVs express CD9.

[Reference] Comparison of CD9 Marker Levels in Healthy Subjects Aged 69 and Under and Those Aged 70 and Over



4.Measurement of platelet markers using EV ELISA

The levels of the EV marker CD9 and the platelet markers CD41 and CD61 were measured in plasma samples from healthy individuals and patients with neurodegenerative diseases (AD and PD) using PS Capture ELISA and antibody sandwich ELISAs. For CD9 measurements, purified EVs derived from COLO201 cells were used as the ELISA standard, whereas purified CD41/CD61-positive EVs isolated from healthy-donor EDTA plasma were used as the ELISA standard for CD41 and CD61 measurements.



The levels of CD41 and CD61, as well as CD9, showed a downward trend in samples from patients with AD and PD compared with those from healthy individuals.

5.Conclusion

These findings indicate that most platelet-derived EVs co-express CD9 and that the levels of CD9 and the platelet markers CD41 and CD61 are reduced in plasma samples from patients with AD and PD. These results suggest that platelet-derived EVs may be reduced in the blood of patients with AD and PD. Previous studies have reported that platelets are involved in the pathogenesis and progression of AD and that morphological and functional abnormalities are observed in platelets from patients with PD. These abnormalities may also affect EV secretion. Future studies could examine platelet-derived EV marker levels in other diseases and further explore their potential as biomarkers for neurodegenerative diseases.