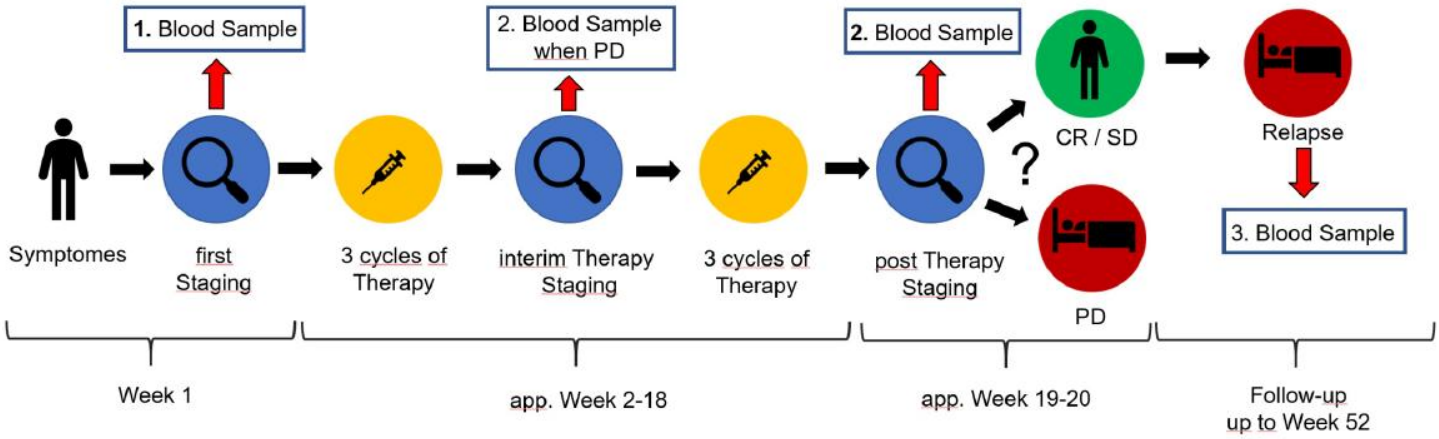
Supplementary Material

Supplemental Table 1: List of commercial protease activity assays used for this study.

Protease	Product Name	Catalog #
ADAM10	SensoLyte ® 520 ADAM10 Activity Assay Kit	AS-72226
ADAM17	SensoLyte ® 520 TACE (α - Secretase) Activity Assay Kit	AS-72085
ADAMTS13	SensoLyte ® 520 ADAMTS13 Activity Assay	AS-72232
Aggrecanase-1	SensoLyte ® 520 Aggrecanase - 1 Assay Kit	AS-72114
BACE1	SensoLyte ® 520 β - Secretase Assay Kit	AS-71144
BACE2	SensoLyte ® 520 BACE2 Activity Assay	AS-72225
Calpain	SensoLyte ® 520 Calpain Activity Assay Kit	AS-72149
Cathepsin B	SensoLyte ® 520 Cathepsin B Assay Kit	AS-72164
Cathepsin D	SensoLyte ® 520 Cathepsin D Assay Kit	AS-72170
Cathepsin E	SensoLyte ® 520 Cathepsin E Assay Kit	AS-72222
Cathepsin G	SensoLyte ® 520 Cathepsin G Assay Kit	AS-72185
Cathepsin K	SensoLyte ® 520 Cathepsin K Assay Kit	AS-72171
Cathepsin L	SensoLyte ® 520 Cathepsin L Assay Kit	AS-72218
Cathepsin S	SensoLyte ® 520 Cathepsin S Assay Kit	AS-72099
Enterokinase	SensoLyte ® 520 Enterokinase Activity Assay Kit	AS-72209
Granzyme A	SensoLyte ® 520 Granzyme A Activity Assay Kit	AS-72260
Granzyme B	SensoLyte ® 520 Granzyme B Activity Assay Kit	AS-72261
IDE/insulysin	SensoLyte ® 520 IDE Activity Assay Kit	AS-72231
Meprin a	SensoLyte ® 520 Meprin α Activity Assay Kit	AS-72253
Meprin b	SensoLyte ® 520 Meprin β Activity Assay Kit	AS-72254
MMP-1	SensoLyte ® 520 MMP - 1 Assay Kit	AS-71150
MMP-2	SensoLyte ® 520 MMP - 2 Assay Kit	AS-71151
MMP-3	SensoLyte ® 520 MMP - 3 Assay Kit	AS-71152
MMP-7	SensoLyte ® 520 MMP - 7 Assay Kit	AS-71153
MMP-8	SensoLyte ® 520 MMP - 8 Assay Kit	AS-71154
MMP-9	SensoLyte ® 520 MMP - 9 Assay Kit	AS-71155
MMP-10	SensoLyte ® 520 MMP - 10 Assay Kit	AS-72024
MMP-12	SensoLyte ® 520 MMP - 12 Assay Kit	AS-71157
MMP-13	SensoLyte ® 520 MMP - 13 Assay Kit	AS-71156
MMP-14	SensoLyte ® 520 MMP - 14 Assay Kit	AS-72025
MMP-generic	SensoLyte ® 520 Generic MMP Activity Kit	AS-71158
Neprilysin	SensoLyte ® 520 Neprilysin Activity Assay Kit	AS-72223
Renin	SensoLyte ® 520 Renin Assay Kit	AS-72040
Thrombin	SensoLyte ® 520 Thrombin Activity Assay Kit	AS-72129

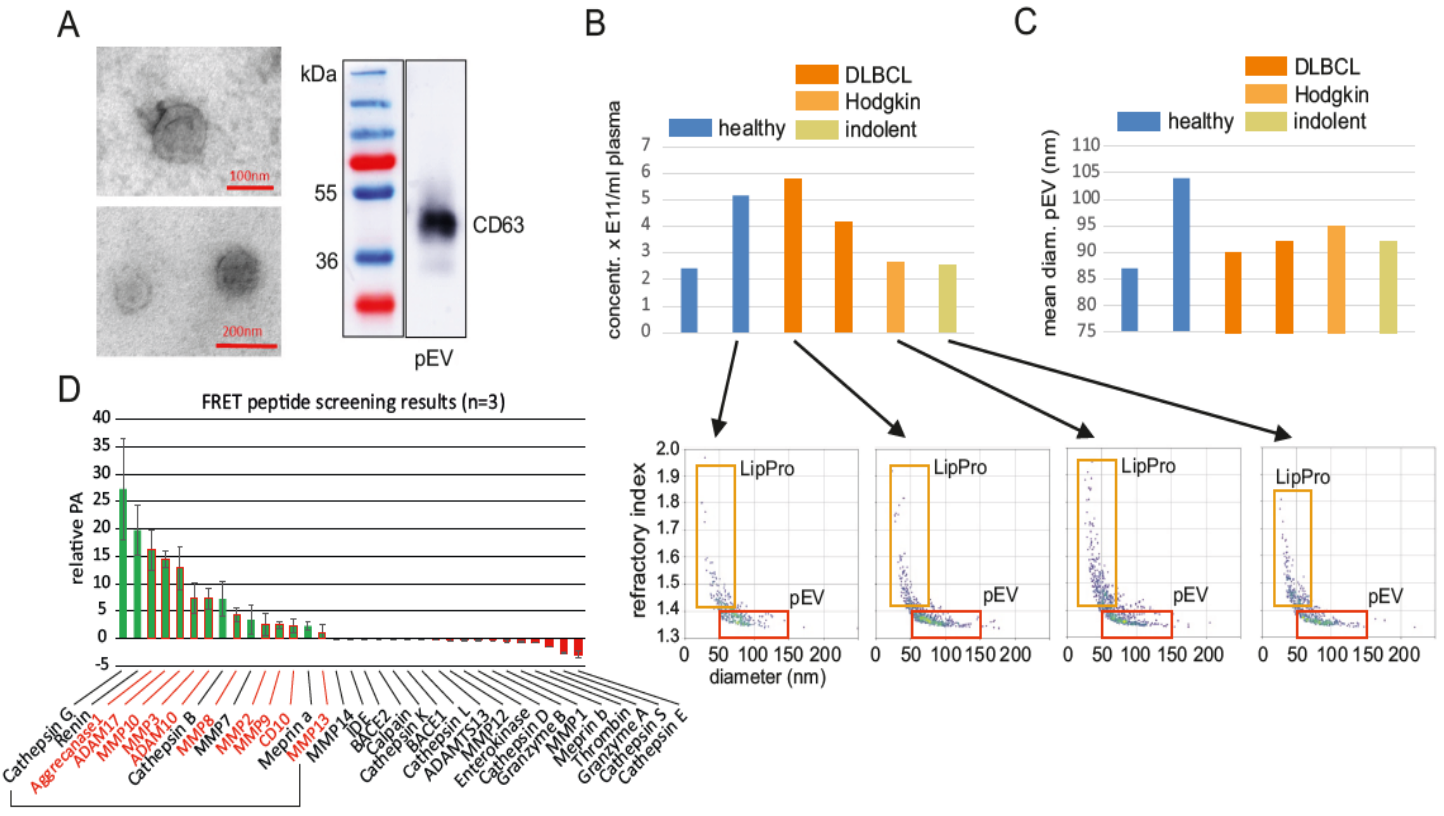
Figure S1. Patient recruitment, clinical staging, treatment, and longitudinal blood sampling.



Patients presenting with suspected lymphoma underwent diagnostic evaluation. Upon confirmation of DLBCL, Hodgkin lymphoma, or indolent lymphoma, a baseline blood sample was collected for pEV analysis (**sample 1**). DLBCL patients were subsequently enrolled for longitudinal pEV analyses during and after six cycles of chemo-immunochemotherapy. A second blood sample was obtained either at interim staging in patients with progressive disease (PD) or at post-therapy staging in patients without progression (**sample 2**). Patients achieving complete remission (CR) or stable disease (SD) at post-therapy staging who developed disease progression during the 52-week follow-up period provided an additional blood sample at progression (**sample 3**).

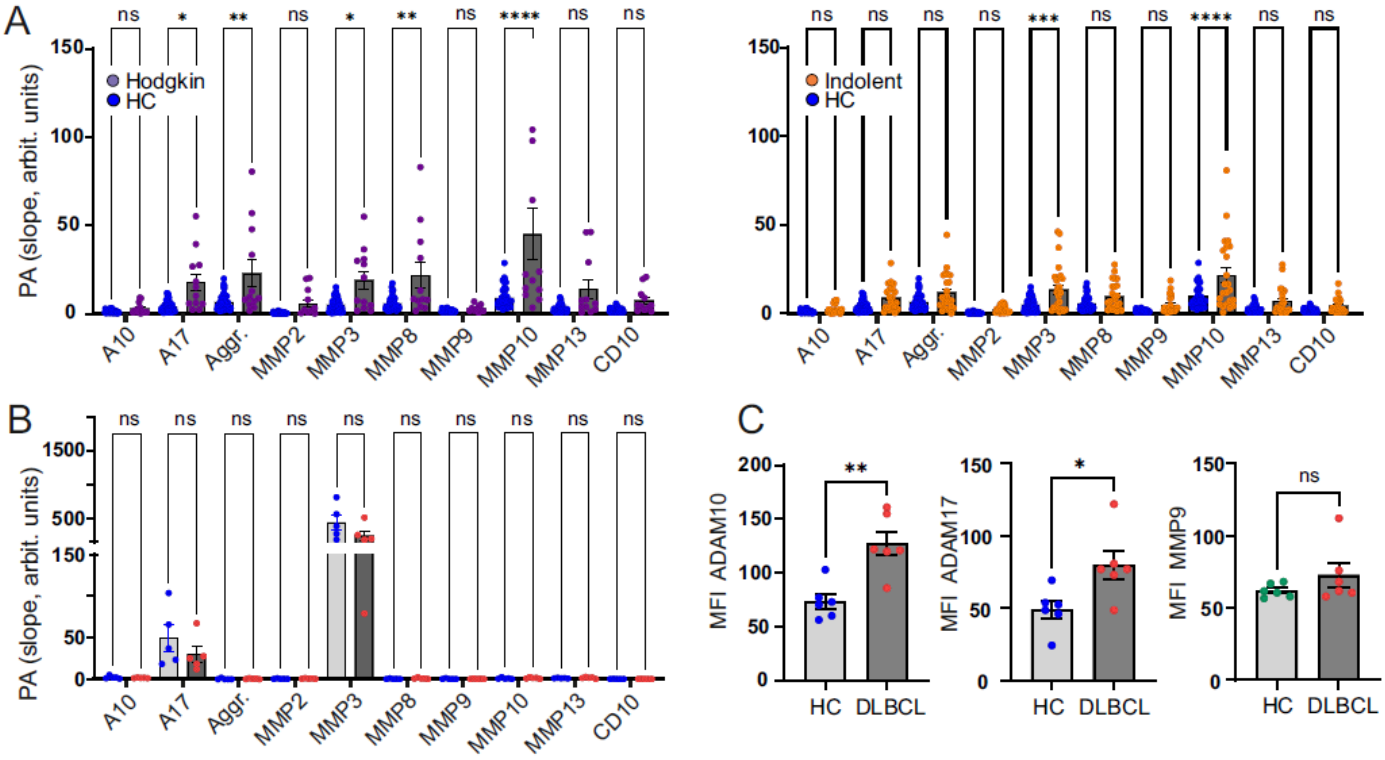
Abbreviations: PD, progressive disease; SD, stable disease; CR, complete remission.

Figure S2. Characterization of pEV and protease-specific FRET peptide cleavage patterns.



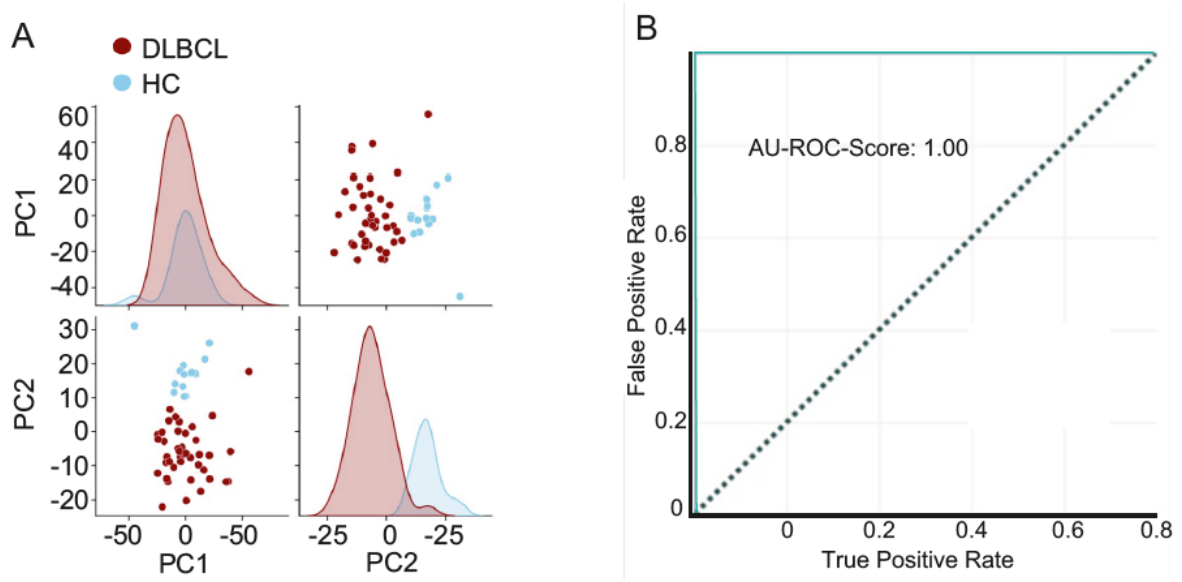
(A) pEVs from patients and healthy controls (HC) display typical size and morphology by transmission electron microscopy (TEM). Expression of tetraspanin CD63 in pEVs was confirmed by western blotting. **(B-C)** Representative pEV samples from HC and lymphoma patients (DLBCL, Hodgkin, and indolent) were analyzed by interferometric nanoparticle tracking analysis (iNTA) for particle concentration **(B)** and size distribution **(C)**. Insets show diameter versus refractive index plots to visualize residual lipoproteins. **(D)** pEV-associated protease activity measured using 33 commercially available FRET peptides in three DLBCL samples. Positive activities (n = 15) are shown in green; negative activities (n = 18) in red. Data are presented as mean ± SD.

Figure S3. pEV-PA in Hodgkin and indolent lymphoma, PA in plasma, and protease presence in pEV.



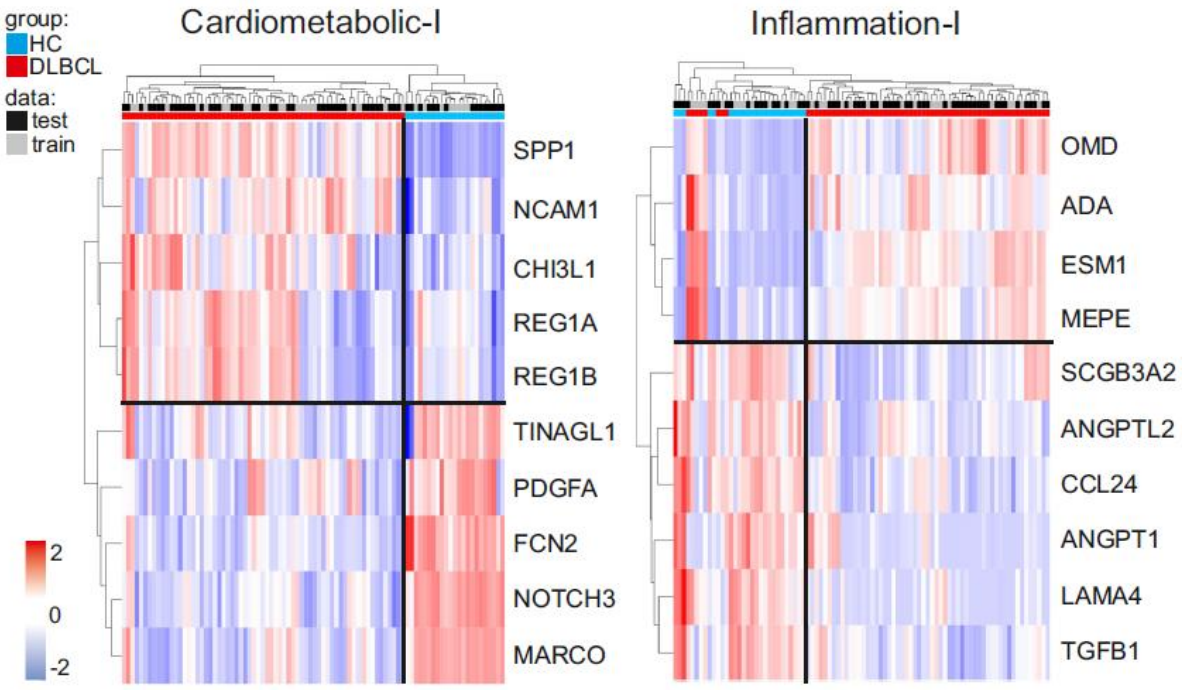
(A) Relative pEV-PA for individual proteases in patients with Hodgkin lymphoma (n = 12, left) and indolent lymphoma (n = 30, right) compared with healthy controls (HC, n = 50).
(B) PA measured directly in unmodified plasma (10 µL) from 5 DLBCL patients across 10 proteases.
(C) Detection of proteases ADAM10, ADAM17, and MMP9 in purified pEV from 6 DLBCL patients and HCs, assessed by flow cytometry (FACS).

Figure S4. Distinction of DLBCL patients from healthy controls using PCA and AU-ROC analysis.



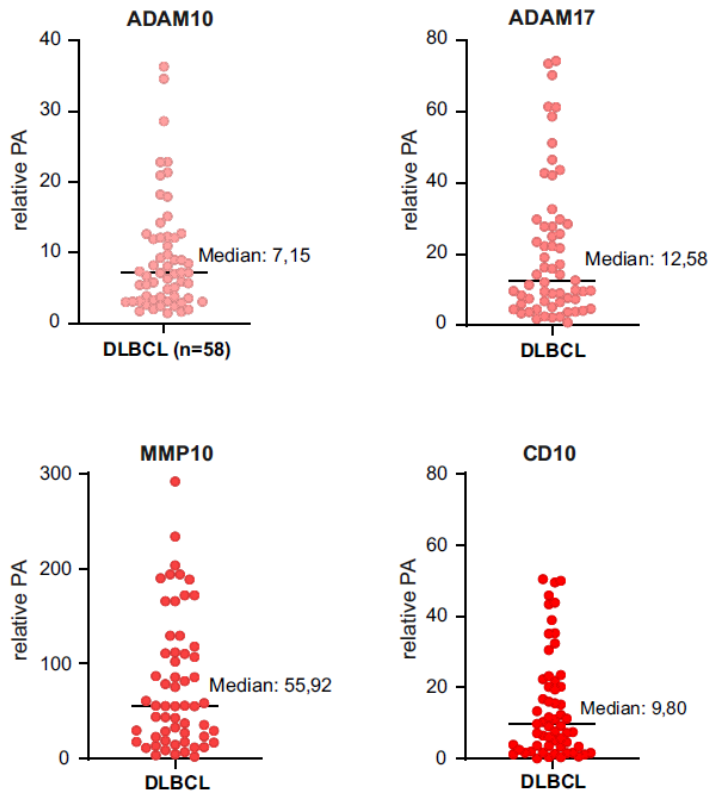
(A) Principal component analysis (PCA) of individual protein profiles from Olink® proteomics (3,000 factors), showing separation between DLBCL patients and HC.
(B) Area under the receiver operating characteristic curve (AU-ROC) for classification of HC versus DLBCL based on the full Olink® 3,000-protein dataset.

Figure S5. Heatmaps of Olink® protein panels Cardiometabolic-I and Inflammation-I.



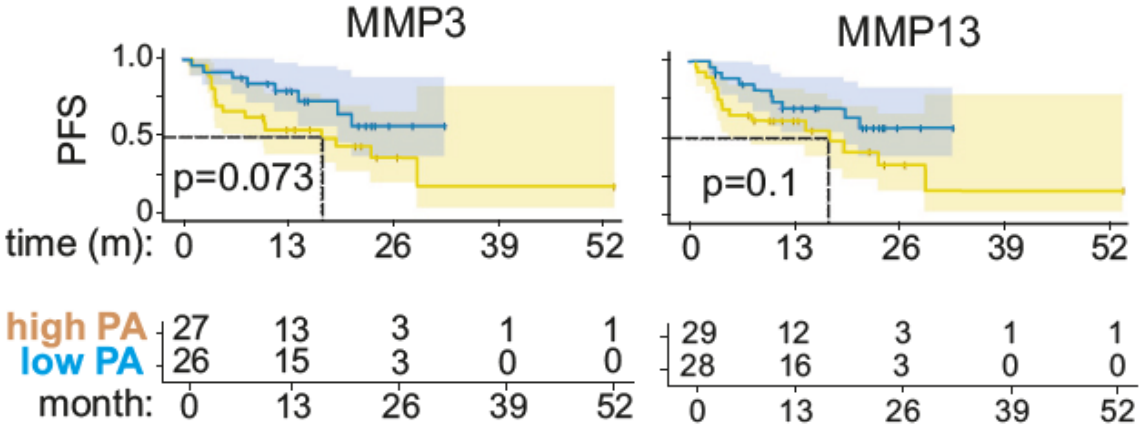
Heatmaps display the top upregulated and downregulated proteins in the Cardiometabolic-I and Inflammation-I panels, as described for **Figure 2C** and illustrated in **Figures 2E** and **2F**.

Figure S6. Median pEV-PAs for ADAM10, ADAM17, MMP10, and CD10 in DLBCL patients.



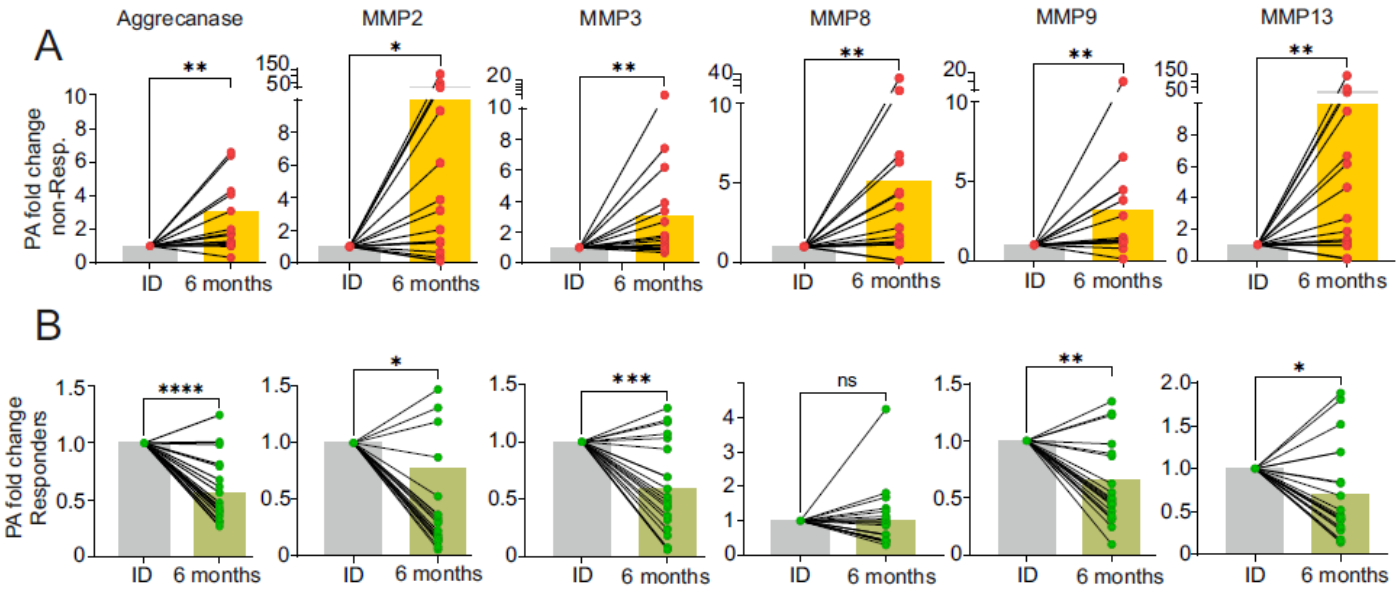
Median values were calculated from individual protease activities presented in **Figure 1C** for patients undergoing treatment.

Figure S7. Kaplan–Meier survival curves stratified by baseline pEV-PAs of MMP3 and MMP13



Patients were categorized into high and low initial PA groups for each protease to evaluate progression-free survival during DLBCL treatment.

Figure S8. Fold changes in pEV-PAs for selected proteases during DLBCL treatment.



Fold decreases or increases in protease activity are shown for responders (green) and non-responders (red). Changes are smaller compared with those observed in **Figure 4C**.