

Supplementary Material

Neoadjuvant Chemo- or Chemo-Radiation-Therapy of Pancreatic Ductal Adenocarcinoma Differentially Shift ECM Composition, Complement Activation, Energy Metabolism, and Ribosomal Proteins of the Residual Tumor Mass

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Supplementary Spreadsheet 1: - Available as separate xlx file.	
Supplementary Spreadsheet 2: - Available as separate xlx file.	
Supplementary Spreadsheet 3: - Available as separate xlx file.	

Table S1: Complete clinicopathological annotation of individuals included in this study.

Sample	Treatment	Chemotherapeutics	Sex	Age	Status	Differentiation	Perineural Invasion	Vascular Invasion	Recurrence	Survival Days #	T stage	N stage	Year
CH.01	Chemo	Folfirinox	F	66	DOD	poorly	Identified	Suspected	No	391	3	1	2016
CH.02	Chemo	Folfirinox	M	67	NED	moderately	Identified	Not Ident.	No	1940	3	0	2016
CH.03	Chemo	Folfirinox	M	67	DOD	poorly	Identified	Identified	No	674	NA	0	2015
CH.04	Chemo	Folfirinox	M	68	DOD	poorly	Identified	Identified	Yes	381	3	1	2016
CH.05	Chemo	Folfirinox	M	69	DOD	moderately	Identified	Identified	Yes	1187	3	0	2016
CH.06	Chemo	Folfirinox/GemZar	F	69	NED	moderately	Identified	Identified	No	1337	3	1	2017
CH.07	Chemo	Folfirinox	M	71	AWD	moderately	Not Ident.	Not Ident.	Yes	1189	1	1	2018*
CH.08	Chemo	Folfirinox	F	72	DOD	moderately	Identified	Identified	Yes	1309	3	1	2016
CH.09	Chemo	Folfirinox	F	73	DOD	moderately	Identified	Not Ident.	Yes	774	3	0	2016
CH.10	Chemo	Folfirinox	M	75	NED	moderately	Identified	Identified	No	1240	3	1	2018*
CH.11	Chemo	Folfirinox	F	76	DOD	moderately	Identified	Not Ident.	Yes	1276	3	0	2017
CH.12	Chemo	Folfirinox	F	79	DOD	moderately	Identified	Identified	Yes	488	2	1	2018*
CH.13	Chemo	Gem/Abraxane	M	80	DOD	moderately	Identified	Identified	Yes	1224	1	1	2018*
CH.14	Chemo	Folfirinox	F	52	DOD	moderately	Not Ident.	Not Ident.	Yes	255	3	0	2017
CH.15	Chemo	Folfirinox	F	72	DOD	well	Identified	Not Ident.	Yes	1161	3	0	2017
CH.16	Chemo	Folfirinox	M	72	DOD	moderately	Identified	Identified	Yes	394	3	1	2016
CH.17	Chemo	Gem	M	82	DOD	moderately	Identified	Identified	Yes	654	3	1	2015
CH.18	Chemo	Folfirinox	F	47	DOD	poorly	Identified	Suspected	No	582	3	0	2015
CH.19	Chemo	Folfirinox	M	85	DOD	poorly	Identified	Identified	No	329	3	1	2016
CH.20	Chemo	Folfox	M	71	DOD	moderately	Identified	Not Ident.	Yes	741	3	1	2016
CH.21	Chemo	Folfirinox	F	33	DOD	poorly to moderately	Identified	Identified	No	374	3	1	2015
CH.22	Chemo	Folfirinox	M	55	AWD	moderately	Identified	Not Ident.	Yes	936	1	0	2018*
CH.23	Chemo	Unknown	F	70	NED	moderately	Identified	Identified	No	255	3	0	2017
CH.24	Chemo	Folfirinox	M	50	DOD	poorly	Identified	Not Ident.	Yes	622	2	0	2017
CH.25	Chemo	Folfirinox	M	55	DOD	poorly to moderately	Identified	Suspected	No	1466	3	0	2015
CH.26	Chemo	Avastin/Gem/Abraxane	F	57	AWD	moderately	Not Ident.	Not Ident.	Yes	1700	1	0	2017
CH.27	Chemo	Folfirinox	M	58	DOD	moderately	Identified	Not Ident.	Yes	325	3	1	2017
CH.28	Chemo	Folfirinox	F	60	DOD	moderately	Identified	Not Ident.	Yes	1688	3	0	2015

CH.29	Chemo	Folfirinox	F	63	DOC	well	Identified	Not Ident.	No	359	3	1	2015
CH.30	Chemo	Folfirinox	M	64	DOD	moderately	Not Ident.	Not Ident.	Yes	1073	3	0	2015
CH.31	Chemo	Folfirinox	M	65	DOD	moderately	Identified	Identified	No	744	2	2	2018*
CH.32	Chemo	Folfirinox	M	66	DOD	moderately	Identified	Identified	No	706	3	1	2015
CH.33	Chemo	Folfirinox	F	71	DOD	moderately	Identified	Identified	No	309	3	1	2017
CH.34	Chemo	Folfirinox	F	73	DOD	moderately	Not Ident.	Not Ident.	Yes	1541	3	0	2015
CH.35	Chemo	Gem/Nab-Paclitaxel	M	80	DOD	moderately	Not Ident.	Identified	No	705	1	1	2018*
CH.36	Chemo	Folfox	M	83	DOD	moderately	Identified	Identified	Yes	584	3	1	2017
CH.37	Chemo	Folfirinox	M	47	DOD	poorly	Not Ident.	Identified	Yes	575	1	1	2016
CH.38	Chemo	Folfirinox	F	50	AWD	poorly	Identified	Identified	Yes	488	2	2	2018*
CR.01	Chemoradiation	Folfirinox/Cap	F	66	DOD	moderately	Not Ident.	Not Ident.	Yes	1424	2	0	2017
CR.02	Chemoradiation	Capecitabine	F	67	DOD	moderately	Identified	Not Ident.	Yes	860	3	0	2013
CR.03	Chemoradiation	Gem	F	68	DOD	moderately	Identified	Not Ident.	Yes	1232	3	0	2012
CR.04	Chemoradiation	Folfirinox/Capecitabine	F	71	DOD	moderately	Not Ident.	Not Ident.	Yes	416	3	0	2017
CR.05	Chemoradiation	Folfirinox	M	75	DOD	moderately	Identified	Identified	Yes	974	1	0	2013
CR.06	Chemoradiation	Folfirinox	M	67	NED	moderately	Identified	Identified	No	1409	2	0	2018*
CR.07	Chemoradiation	Gem/Xerloda	F	68	DOD	moderately	Identified	Not Ident.	Yes	641	3	0	2012
CR.08	Chemoradiation	Capecitabine	F	77	DOD	moderately	Identified	Not Ident.	Yes	264	3	0	2012
CR.09	Chemoradiation	Folfirinox	M	72	DOD	moderately	Identified	Not Ident.	Yes	612	3	0	2013
CR.10	Chemoradiation	Gem	M	66	DUC	moderately	Identified	Identified	No	581	3	1	2013
CR.11	Chemoradiation	Gem/Zerloda	M	56	NED	moderately	Identified	Not Ident.	No	3496	3	0	2012
CR.12	Chemoradiation	Folfirinox/Xerloda	F	45	DOD	moderately	Identified	Identified	Yes	482	3	0	2017
CR.13	Chemoradiation	Folfox	M	61	DOD	moderately	Identified	Not Ident.	Yes	895	3	0	2017
CR.14	Chemoradiation	Unknown	M	64	DOD	moderately	Identified	Identified	Yes	689	3	0	2017
CR.15	Chemoradiation	Gem/Paclitaxel	F	74	NED	moderately	Identified	Not Ident.	No	1454	3	1	2017
CR.16	Chemoradiation	Folfirinox	M	76	DOD	moderately	Not Ident.	Identified	Yes	424	2	0	2018*
CR.17	Chemoradiation	Folfirinox/Capcutabine/Gem	F	48	NED	moderately	Identified	Not Ident.	No	3407	2	0	2012
CR.18	Chemoradiation	Folfirinox	M	49	DOD	poorly to moderately	Identified	Not Ident.	No	676	3	0	2015
CR.19	Chemoradiation	Folfox/Zerloda	M	80	AWD	poorly	Identified	Not Ident.	Yes	1274	3	1	2018*
CR.20	Chemoradiation	Folfirinox/Gem	M	58	AWD	moderately	Not Ident.	Not Ident.	Yes	3083	3	0	2013
CR.21	Chemoradiation	Folfirinox	F	58	DOC	moderately	Identified	Identified	No	292	3	1	2014

CR.22	Chemoradiation	Folfinirinox	M	59	AWD	poorly	Identified	Identified	Yes	484	3	1	2017
CR.23	Chemoradiation	Folfinirinox/Gem	F	59	DOD	moderately	Identified	Not Ident.	Yes	2836	3	0	2013
CR.24	Chemoradiation	Folfox/Gem	M	60	NED	poorly to moderately	Identified	Not Ident.	No	2428	3	0	2015
CR.25	Chemoradiation	Folfinirinox/Zerloda	F	60	NED	moderately	Not Ident.	Not Ident.	No	1663	1	0	2017
CR.26	Chemoradiation	Gem/Oxalip/Zerloda	F	62	DOD	moderately	Not Ident.	Not Ident.	Yes	1049	1	0	2012
CR.27	Chemoradiation	Folfinirinox	M	65	DOD	moderately	Identified	Not Ident.	Yes	481	3	0	2013
CR.28	Chemoradiation	Folfinirinox	F	65	DOD	moderately	Identified	Not Ident.	Yes	2124	3	1	2015

AWD: alive with disease, DOC: died of other cause, DOD: died of disease, DUC: died of unknown cause, NED: no evidence of disease

* Classification based on the 8th edition of the AJCC Cancer Staging Manual. All cases before 2018 were classified based on the 7th edition.

Survival was calculated starting from the first day of diagnosis.

Table S2: Transcriptomic RNA-Seq data for proteogenomic analysis. Transcriptomic data were downloaded from the SRA database on NCBI.

SRA Identifier	Link to corresponding SRA File
SRR16201987	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16201987
SRR16201988	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16201988
SRR16201989	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16201989
SRR16201990	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16201990
SRR16201992	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16201992
SRR16201994	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16201994
SRR16202000	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16202000
SRR16202002	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16202002
SRR16202004	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16202004
SRR16202006	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16202006
SRR16202008	https://www.ncbi.nlm.nih.gov/sra/?term=SRR16202008

Table S3: Prognostic Candidate Marker Proteins after Neoadjuvant Treatment

Neoadjuvant treatment	CoxBoost coefficient*	Uniprot ID	Protein name
Chemo-radiation	-0.147	P43652	Afamin
	0.087	P28062	Proteasome subunit beta type-8 (PSMB5i)
Chemo	-0.045	Q9UM54	Unconventional myosin-VI
	-0.042	P05164	Myeloperoxidase

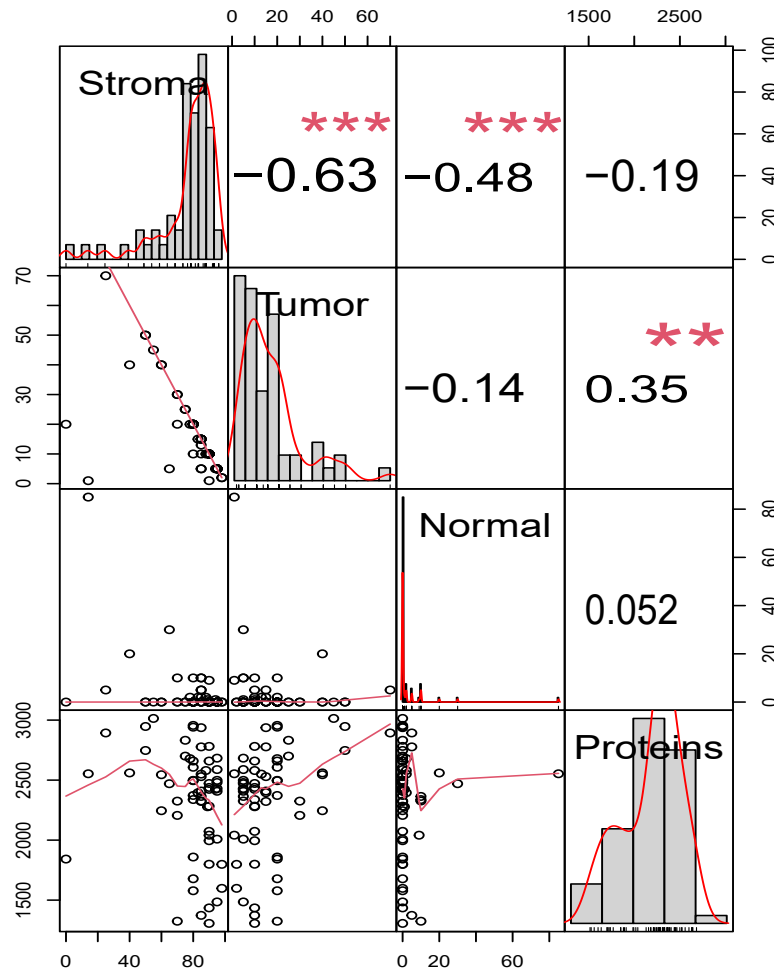
* Coxboost coefficients at boosting step = 30; Coefficient < 0.04 = high protein expression is favorable; Coefficient > 0.04 = high protein expression is unfavorable.

Table S4: List of the 13 most frequent SAAVs, which are present in at least 50 % of all proteomic samples.

Protein ID	Protein Name	SAAV	ClinVar: Variation Annotation	ClinVar: Clinical Significance
O60504	Vinexin	I5556T	-	-
P01619	Immunoglobulin kappa variable 3-20	S74N	-	-
P02751	Fibronectin	V2114I	NM_212482.4(FN1):c.6781G>A (p.Val2261Ile)	Benign (Dec 18, 2021)
P06727	Apolipoprotein A-IV	S147N	NM_000482.4(APOA4):c.440G>A (p.Ser147Asn)	Benign (Dec 18, 2021)
P07942	Laminin subunit beta-1	Q1022R	NM_002291.3(LAMB1):c.3065A>G (p.Gln1022Arg)	Benign (Dec 18, 2021)
P12110	Collagen alpha-2(VI) chain	R680H	NM_001849.4(COL6A2):c.2039G>A (p.Arg680His)	Benign (Dec 19, 2021)
P30084	Enoyl-CoA hydratase, mitochondrial	T75I	NM_004092.4(ECHS1):c.224C>T (p.Thr75Ile)	Benign (Dec 19, 2021)
P32455	Guanylate-binding protein 1	T349S	Protein not found on ClinVar.	
Q01518	Adenylyl cyclase-associated protein 1	S256A	-	-
Q16666	Gamma-interferon-inducible protein 16	R409S_Q413N	-	-
Q5JTV8	Torsin-1A-interacting protein 1	M146T	NM_015602.4(TOR1AIP1):c.437T>C (p.Met146Thr)	Benign (Sep 10, 2021)
Q969G5	Caveolae-associated protein 3	R8P	-	-
Q96HC4	PDZ and LIM domain protein 5	S383N	-	-

- AAV not found in ClinVar database

(A)



(B)

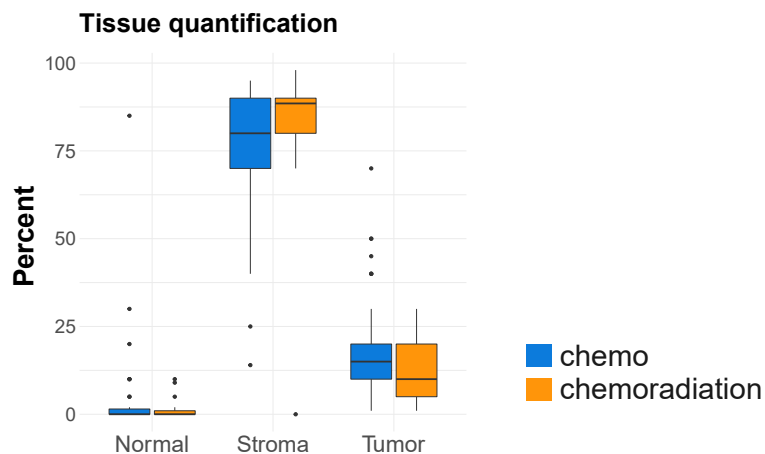


Fig S1: (A) Pearson correlation analysis between the number of identified proteins and the percentages of normal, tumor and stromal tissue revealed that there is only a low correlation between the number of identified proteins and the tissue composition. The boxes in the lower left panel show the distribution of the corresponding values of two conditions indicating potential correlation. The number of identified proteins shows a positive but weak correlation with the percentage of tumor tissue (correlation coefficient = 0.35), while it correlated negative but also weak with the percentage of stroma tissue (correlation coefficient = -0.19) in a sample. (B) Quantification of the amount of tumor, stroma and normal tissue per condition.

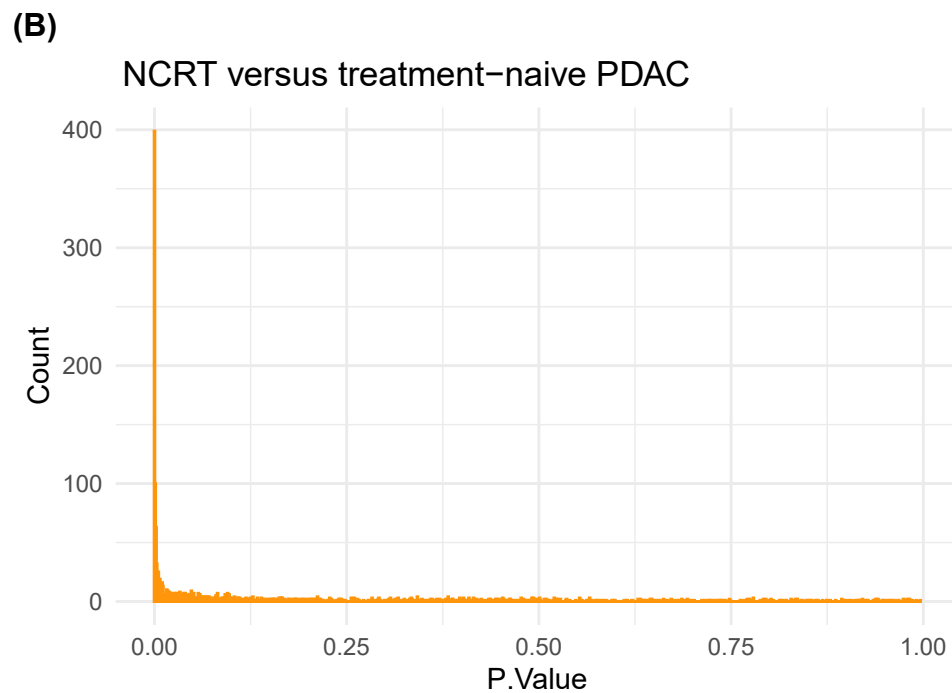
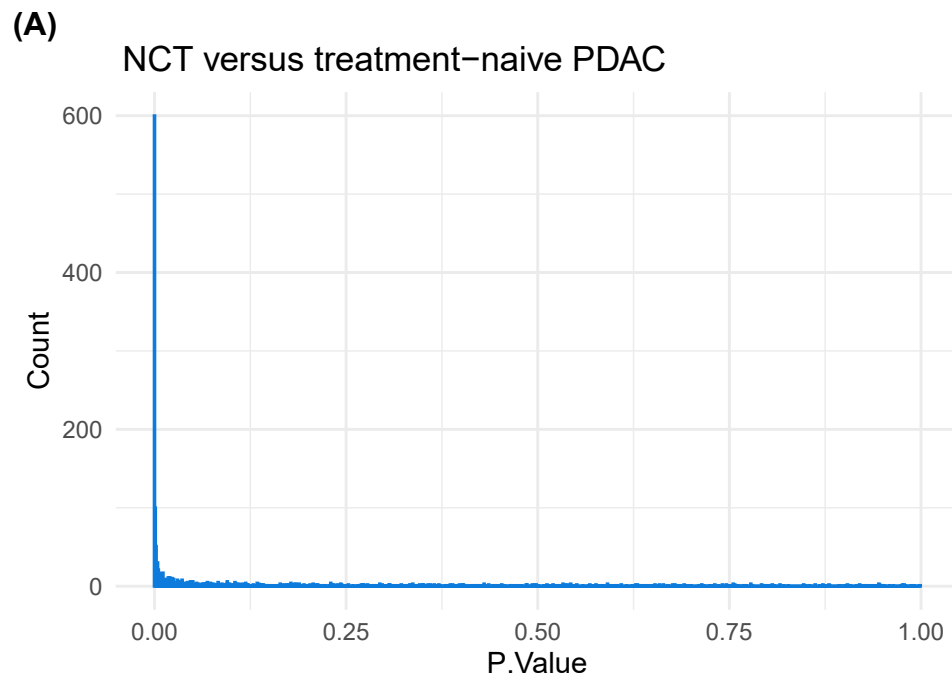
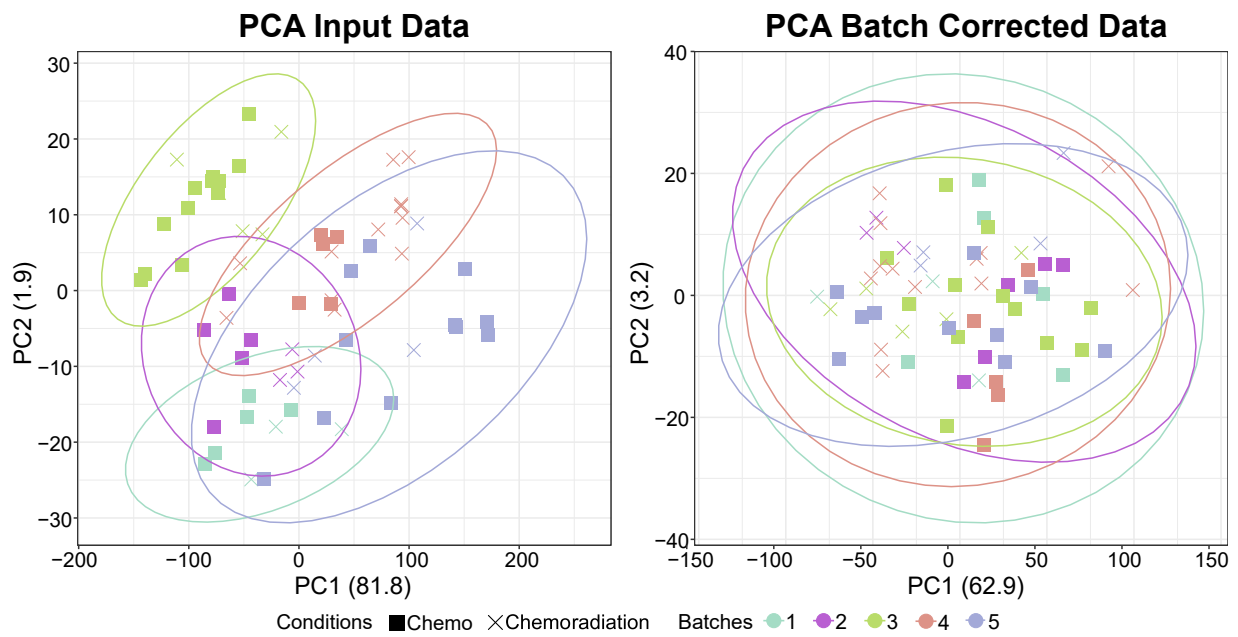


Fig. S2: (A) Histogram presenting the distribution of p values of proteins in the comparison neoadjuvant chemotherapy (NCT) versus treatment-naïve PDAC, and (B) the comparison of neoadjuvant chemo-radiation (NCRT) versus treatment-naïve PDAC.

(A)



(B)

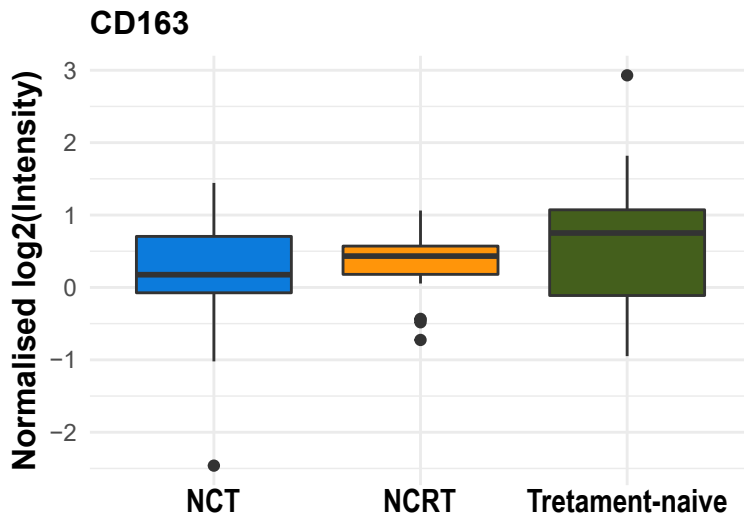


Fig. S3: (A) Principal Component Analysis (PCA) of unprocessed input (left) and batch corrected (right) data using the ComBat algorithm. Batch correction was applied on the NCRT and NCT samples according to the sample preparation batch. (B) Protein expression of macrophage polarization marker CD163.

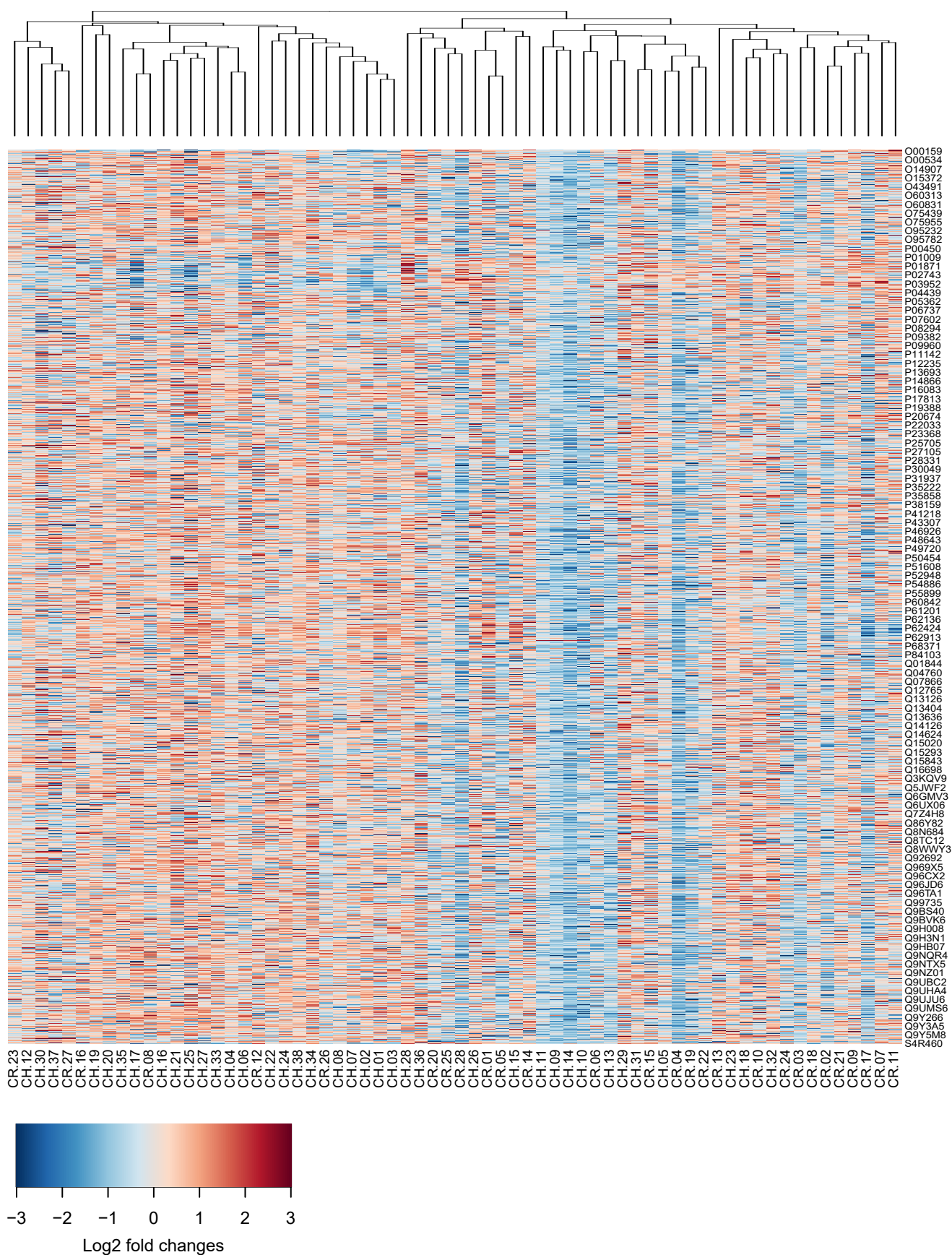


Fig. S4: Hierarchical clustering of all quantified proteins and samples revealed no group clustering of samples. CH = chemo, CR = chemo-radiation.

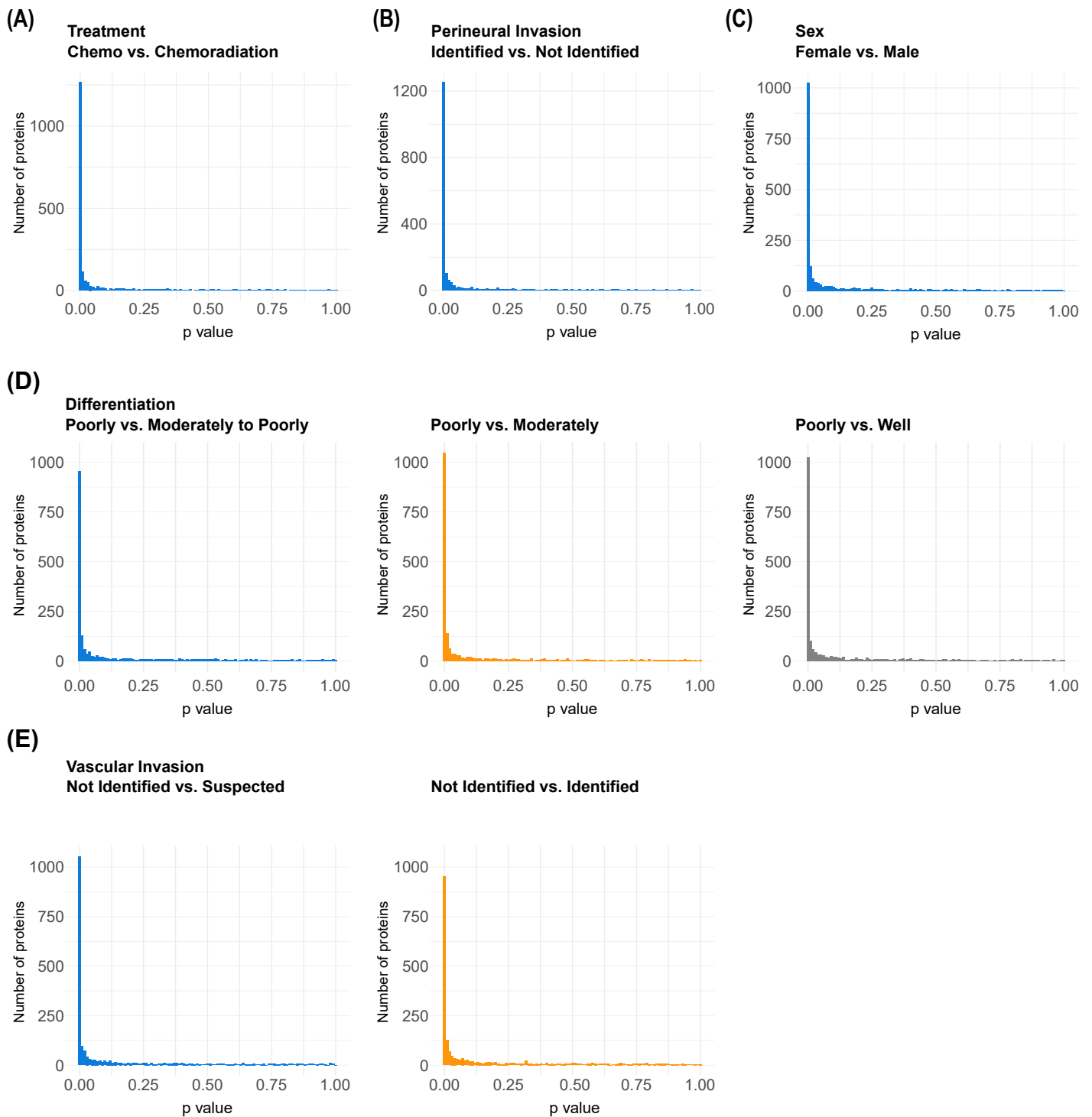
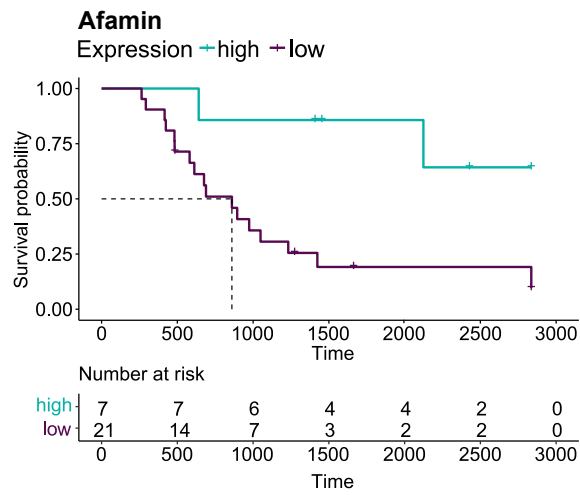


Fig. S5: Proteomic comparison between NCT and NCRT, and influence of clinical and histopathological co-variables after NCT (n=38) and NCRT (n=28). (A-E) Histograms presenting the distribution of p values of proteins affected by clinical and histopathological factors. All variables show a large number of proteins that present p-values below 0.05 in the DEA.

(A)



(B)

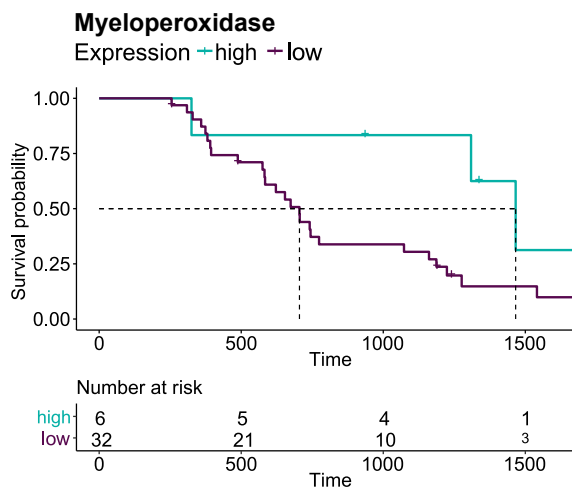


Fig. S6: (A) Overall survival probability of NCRT patients with high (turquoise) and low (purple) protein expression of AFM. (B) Overall survival probability of NCT patients with high (turquoise) and low (purple) protein expression of MPO.