Proteome landscape of B-cell malignancies identifies mantle cell lymphoma protein signature

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CONFLICTS OF INTEREST

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ABSTRACT

Mantle cell lymphoma (MCL) is one of the deadliest forms of Non-Hodgkin's B-cell lymphoma. Typically, patients present with both overexpression of CyclinD1 and secondary mutations identified by genomic sequencing. Although MCL patients may initially respond to treatment, they eventually relapse and succumb to disease, highlighting the essential need to identify new targets for treatment. Here we performed proteomic profiling of healthy B cells and three different forms of B-cell malignancies, including MCL, to define the proteomic signature of MCL. We compared the proteome of each to MCL and identified 10 proteins that are specifically upregulated in MCL. Of these 10 proteins, seven of them show no transcriptional changes and have been overlooked by conventional RNA expression analysis. Further analysis of the proteomic signature reveals potential avenues for dual targeting in CAR T-cell therapy and provides guidance for personalized therapeutics based on protein expression.

STATEMENT OF SIGNIFICANCE

We present a resource defining the protein landscape of MCL, CLL, and FL as compared to healthy b cells identified utilizing quantitative proteomics from primary patient samples. Applied to MCL, our results identify 10 proteins specifically upregulated in MCL that may prove to be therapeutic targets to treat the disease.

65 **INTRODUCTION**

66 Malignancies affecting the hematopoietic system make up approximately 8-10%
67 of all human cancers.[1] The two most common adult hematopoietic malignancies in
68 western countries are chronic lymphocytic leukemia (CLL) and follicular lymphoma (FL).
69 Both are B-cell malignancies with incidences of 25-30% of all adult leukemias and 20-
70 40% of all Non-Hodgkin's Lymphomas (NHL), respectively.[2] Current therapies have
71 improved treatment options and overall survival (OS). CLL has an ~87% 5-year OS, in
72 part due to the BTK inhibitor Ibrutinib being approved by the FDA in 2016 for frontline
73 treatment.[3] Likewise, FL has an ~90% 5-year OS associated with the addition of the
74 monoclonal antibody rituximab being used in conjunction with chemotherapeutics.[4]

75 Unfortunately, not all B-cell malignancies have similar survival rates. In the case
76 of mantle cell lymphoma (MCL), an aggressive NHL that accounts for ~4-6% percent of
77 all lymphoma cases in the United States, patients have only an ~40% 5-year survival.[2]
78 Besides the standard high dose chemotherapy regimen, limited treatments are available,
79 resulting in poor outcomes.[2] Recently, chimeric antigen receptor T-cell (CAR-T) therapy
80 targeting the B-cell marker CD19 has been approved by the FDA for treatment in
81 relapse/refractory MCL.[5] Although this provides a new therapeutic option for MCL
82 patients, it is not considered a universal cure and many patients still relapse.[5] Additionally,
83 the proteasome inhibitor Bortezomib was approved for MCL patients with relapse or
84 refractory disease in 2013; however, discrepancies between survival of these patients
85 show a significant need for new first-line therapeutics.[6]

86 This study aims to garner a better understanding of MCL pathogenesis and create
87 a resource that can be used for the identification of potential novel therapeutic targets to

88 improve patient prognosis. Currently, most research has focused on gene expression
89 profiling (GEP) of patients to identify molecular predictors of disease; however, these
90 studies are not extensively validated at the protein level where targeted therapies take
91 effect. Additionally, current proteomic studies are heavily reliant on cell lines which cannot
92 accurately mimic patient tumors.[7, 8] When patient samples have been utilized, the
93 comparison has only been made to healthy donor (HD) and not between disease types.[9,
94 10] The one instance where disease types have been compared (MCL to CLL), only
95 transcriptomics was utilized.[11] To address this gap in knowledge, we utilized 35 primary
96 patient samples to identify an MCL-specific proteomic signature by comparing MCL to
97 CLL, FL, and HD B cells by quantitative mass spectrometry. The minimal overlap
98 observed between proteomic and GEP analyses emphasizes the importance of
99 developing such a resource. Of particular interest are proteins we have identified as
100 differentially expressed on the plasma membrane, as these may be ideal targets for CAR-
101 T therapy. We also identified patient-specific variations in the protein abundance of
102 proteasomal subunits suggesting that addition of Bortezomib to the treatment regimen
103 may benefit some patients, but not others. Finally, our results identify 10 proteins
104 specifically upregulated in MCL. Of these 10 proteins, seven are unchanged at the mRNA
105 level, and would therefore not be identified by GEP. Importantly, three of these proteins
106 have never been associated with any lymphoid malignancy, making them a novel finding.
107
108 **RESULTS**

109 **Proteomics signatures of MCL, CLL, and FL**

110 To identify the protein landscape of MCL, CLL, and FL, we performed quantitative
111 tandem mass tag (TMT) mass spectrometry on B cells from 5 HD, 7 CLL, 7 FL, and 16

112 MCL patients (**Supplemental Table S1**). We identified 8,027 proteins in total with 1,502
113 proteins present in all batches (**Figure 1A and B**, **Supplemental Table S2**). Principal
114 component analysis (PCA) shows that HD and individual malignancies cluster among
115 themselves and lymphomas capable of forming solid tumors (MCL and FL) cluster
116 similarly while CLL, which remains predominantly in the peripheral blood, clusters
117 separately (**Figure 1C**).

118 Heat map of all disease protein abundances shows that samples cluster by
119 disease sub-type and form five distinct protein clusters associated with various Gene
120 Ontology (GO) pathways (**Figure 1D, Supplemental Tables S3-S8**). The most distinct
121 clusters being one, four, and five. Cluster one proteins, upregulated in MCL and FL,
122 primarily contain proteins associated with the immune response, but also contain proteins
123 associated with cell adhesion and angiogenesis. Because these tumors can infiltrate
124 secondary lymphoid tissues to form solid tumors, targeting these proteins may be
125 significant for slowing disease progression. Cluster four proteins, upregulated in CLL, are
126 associated with mitochondrial health and function. It has been reported that CLL cells are
127 heavily reliant on mitochondrial metabolism.[12] Therefore, proteins within this cluster may
128 prove to be vulnerabilities within the disease ideal for targeting. Cluster five proteins are
129 downregulated in FL and MCL and are associated with RNA processing. We have
130 previously reported that B cells lacking the catalytic domain of the E3 ubiquitin ligase
131 component *n*-recognin 5 (UBR5) have splicing defects.[13] Given that UBR5 is mutated in
132 ~18% of MCL patients, mutations to UBR5's catalytic domain are specific to MCL tumors,
133 and UBR5 is the second most mutated gene in our MCL patient cohort, present in 31%
134 of samples, proteins in this cluster may provide insight into how UBR5 mutations affect

135 MCL pathogenesis (**Supplemental Figure S1A**).[14, 15]

136 As compared to HD, there are 131 significantly dysregulated proteins (104
137 upregulated, 27 downregulated) in MCL, 101 in CLL (70 up, 31 down), and 152 in FL (131
138 up, 21 down) (**Figure 1E, Supplemental Table S9**). Of the dysregulated proteins, only 6
139 are consistently upregulated and 3 are consistently downregulated across all three
140 malignancies. GO analysis of dysregulated proteins in each malignancy identifies unique
141 and similar pathways between each lymphoma type (**Figure 1F**). As discussed above,
142 both MCL and FL have upregulation of proteins associated with cell-cell adhesion,
143 angiogenesis, and inflammatory response. Proteins of interest include intercellular
144 adhesion molecule 1 (ICAM1) and transforming growth factor β-induced protein (TGFBI).
145 (**Figure 1G, orange**). High ICAM1 surface levels in FL and MCL, but low levels in CLL is
146 linked to unfavorable patient prognosis.[16] Although it has not been studied in MCL,
147 transcriptional silencing of TGFBI is considered to be a potential mechanism of B-cell
148 transformation resulting in FL.[17] These data are in line with our proteomics data, validating
149 our findings.

150 Upregulated protein pathways unique to MCL are actin filament organization and
151 positive regulation of cell migration. They include SH3-domain binding protein 1
152 (SH3BP1) and myristoylated alanine-rich C-kinase substrate (MARCKS) (**Figure 1G,**
153 **purple**). While upregulation of SH3BP1 is a new finding in lymphoma, it's upregulation is
154 associated with poor prognosis in acute myeloid leukemia (AML) patients.[18] *MARCKS* is
155 upregulated in MCL and has an important role in cancer spread, invasion, metastasis,
156 and Bortezomib resistance in multiple myeloma (MM).[11]

157 FL has one unique upregulated pathway, signal transduction, that is associated

158 with 19 upregulated genes. Of these genes, four of them are specific to FL: Rho GTPase-
159 activating protein 1 (ARHGAP1), complement component c3 (C3), Ras-related protein
160 Rap-1b (RAP1B), and guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit Beta-2
161 (GNB2) (**Figure 1G, pink**). The most significant of these proteins, ARHGAP1, has not
162 previously been associated with lymphoma; however, one study found that it is
163 alternatively spliced in a TRAF6 dependent manner in myelodysplastic syndrome.[19]

164 Upregulated pathways in CLL are very different from MCL and FL. Some proteins
165 associated with these pathways include glutathione peroxidase 1 (GPX1) and thiol S-
166 methyltransferase (METTL7A) (**Figure 1G, light blue**). High GPX1 expression correlates
167 with poor prognosis in lymphoma patients.[20] METTL7A has not previously been
168 associated with lymphoma but overexpression is associated with enhanced human bone
169 marrow stem cell viability.[21]

170 Pathways downregulated in each malignancy are highly unique. Some potentially
171 relevant downregulated proteins from each malignancy include methyl-CpG-binding
172 protein 2 (MECP2) in MCL, tyrosine-protein phosphatase non-receptor type 6 protein
173 (PTPN6) in FL, and cluster of differentiation 22 (CD22) in CLL (**Figure 1G, light green**).
174 MECP2 interacts with the SIN3a/histone deacetylase 1/2 corepressor complex to silence
175 BIM, a proapoptotic member of the BLC-2 family.[22] PTPN6 is an important repressor of
176 STAT3 activation, a transcription factor constitutively activated in a variety of cancers,
177 including lymphoma, and is considered a tumor suppressor in AML and MM.[23]

178 Overall, this data identifies similarities and unique aspects of three different types
179 of lymphoid malignancies, which have been previously unidentified or underexplored.

180 These pathways and proteins may prove to be prognostic markers for their respective
181 diseases and highlight potential vulnerabilities for possible treatment strategies.
182
183 **Identification of cell surfaceome**

184 Recent success of CAR-T therapy in B-cell malignances targeting specific cell
185 surface receptors reinforces the importance of the cell surfaceome. Of the 1,502 proteins
186 identified in our MS analysis, 120 of them localize to the plasma membrane (**Figure 2A**).
187 One of the defining characteristics of CLL is that it is $CD5^+$, $CD19^+$, and $CD23^+$ with weak
188 amounts of CD22, CD79b, and surface immunoglobulin and is $FMC7^-$.[24] FL cells are
189 $CD19^+$, $CD20^+$, $CD10^+$, and $BCL\text{-}6^+$, but are $CD5^-$ and $CD23^-$.[25] MCL cells are
190 characterized by B-cell surface markers $CD19^+$, $CD20^+$, $CD22^+$, $CD79a^+$, $CD5^+$, $FMC\text{-}7^+$,
191 and $CD10^-$.[26] Cell surface protein pattern of CD5, CD19 and CD22 was consistent with
192 diagnostic markers (**Figure 2B**). Because CAR-T therapy is rapidly becoming the
193 treatment of choice for patients that stop responding to other therapies, and the high
194 incidence of relapse in lymphoid malignancies – MCL has a 100% relapse rate following
195 initial treatments – plasma membrane proteins may prove to be new targets for CAR-T
196 treatment. Of these 120 proteins, 48 of them are either significantly up- or downregulated
197 in each of the three malignancies individually as compared to HD B cells (**Figure 2C**). Of
198 these 48 proteins, 11 of them are specifically upregulated in MCL (**red text**), 8 in FL
199 (**green text**), and 7 in CLL (**dark blue text**). 10 of these proteins overlap between MCL
200 and FL (**orange text**), 1 overlaps between CLL and FL (**light blue text**), and two are
201 upregulated between all three malignancies (**purple text**). We measured abundance of
202 cell surface proteins identified in our analysis in MCL and CLL cell lines – Jeko1 and
203 Granta519 (MCL) and HG3 (CLL) – and compared them to peripheral blood in HD (**Figure**

204 **2D**). We found that in these cell lines, some proteins, such as LGALS9, CD81, and ICAM1
205 matched our proteomic findings, while others such as CD5, CD22, and B2M were more
206 variable. These differences are likely due to differences between the *in vitro* and *in vivo*
207 microenvironments, emphasizing the importance of utilizing patient samples when
208 identifying therapeutic targets of interest.

209 Current CAR-T therapy in lymphoma targets $CD19^{+}$ cells.[5] It has been suggested
210 that low CD19 may diminish efficacy of CAR-T treatment suggesting dual receptor
211 targeting could improve outcomes.[27] We found that CD81 was increased in MCL, but not
212 CLL. When we stratified MCL patients based on CD19 expression, we found that CD19
213 and CD81 had an inverse relationship to one another, but no significant difference in
214 CD22 expression (**Figure 2E and 2F**). This suggests that using CD81 as a CAR-T therapy
215 target in combination with CD19 may improve patient outcomes. Together, these findings
216 provide additional potential receptors for cellular therapy.

217

218 **Genetic heterogeneity of MCL corresponds to proteomic heterogeneity**

219 It is thought that genetic heterogeneity is one of the reasons for the poor survival
220 and limited treatments in MCL.[14] The 16 MCL patients included in our study had typical
221 age and male to female distribution. While there were mutations in 88 different genes,
222 with patients having up to 15 mutations, we saw the expected frequency in MCL for TP53
223 and ATM mutations (**Supplemental Figure S1A**). PCA analysis revealed no distinct
224 clustering in the MCL samples (**Figure 3A**). Overall mutational profile or number of
225 mutations again revealed no specific clustering in the MCL cohort (**Supplemental Figure**
226 **S1B**).

227 Besides standard high dose of cytarabine regime, limited treatments are available
228 for patients with MCL, resulting in poor survival rates and outcomes.[2] The proteasome
229 inhibitor Bortezomib has recently been approved for patients with relapse or refractory
230 disease, however, there has been mixed results.[6] It has been reported that expression of
231 the proteasome subunits correlates with proteasome activity and increased sensitivity to
232 proteosome inhibition.[28] Clustering MCL, CLL, and FL patients based on protein
233 expression of proteasome components shows diverse expression profiles among the
234 MCL cohort (**Figure 3B**). A number of patients clustered with HD samples with low
235 expression of proteasome components (MCL-006, MCL-003, and MCL-038) suggesting
236 that the patients would have decreased sensitivity to Bortezomib. These findings suggest
237 that for a heterogenous disease like MCL, precision targeted therapeutics could benefit
238 patients and highlight the importance of using proteomics to identify new therapies that
239 may improve patient outcomes.
240
241 **Novel post transcriptionally regulated proteins in MCL**
242 Because MCL has the lowest 5-year survival and a high need for new therapeutics,
243 we compared protein expression of MCL patients to both HD B cells and to the other B-
244 cell malignancies. While there were no proteins consistently downregulated in MCL as
245 compared to all groups, comparison of the upregulated proteins found that there are 10
246 proteins specifically upregulated in MCL (**Figure 4A and B, Supplemental Table S10**).
247 These proteins are high mobility group box 3 (HMGB3), EH domain containing 1 (EHD1),
248 MARCKS, galectin 9 (LGALS9), PDZ and LIM domain protein 1 (PDLIM1), drebrin 1
249 (DBN1), glutathione S-transferase Pi (GSTP1), dimethylarginine dimethylaminohydrolase
250 2 (DDAH2), stathmin 1 (STMN1), and four and a half LIM domains protein 1 (FHL1).

251 Importantly, all 10 of these proteins had 3 or more unique peptides identified.
252 (**Supplemental Table S2, Supplemental Figure S2)**. Of these, seven have never been
253 associated with MCL and three have never been associated with any form of lymphoid
254 malignancy (**Supplemental Figure S2**). Using previously published transcriptional data
255 that includes these same MCL patients, we compared mRNA expression for these 10
256 proteins.[29] We found that seven of them are unchanged transcriptionally (**Figure 4C**). The
257 RNA expression level of these 10 genes shows no significant difference in patient
258 survival, which is not surprising given that the transcripts are unchanged (**Supplemental**
259 **Figure S3**).

260 To validate the upregulation of these proteins in MCL, we performed western blots
261 for three these 10 proteins (STMN1, GSTP1, and HMGB3) in both primary patients and
262 patient-derived cell lines including three MCL cell lines: Mino, Jeko1, and Granta519; two
263 CLL cell lines: Mec1 and HG3; and two other lymphoma cell lines: REH, a B-cell acute
264 lymphoblastic leukemia (B-ALL) cell line; and Ramos, a Burkitt's lymphoma cell line
265 (**Figure 4D and E)**. Our results in both models show these proteins are increased in MCL
266 as compared to HD B cells and to samples from other lymphoma types.

267 These findings identify novel proteins that are differentially expressed at the protein
268 level that have previously not been identified in classic transcriptional profiling, suggesting
269 potential novel targets for drug discovery or new translational avenues for PROTAC-
270 based drug development tailored to highly expressed proteins in MCL.

271
272 **DISCUSSION**

273 Historically, therapeutic targets for cancer treatments have been identified using
274 GEP. While these studies have identified many relevant targets, many of them have not

275 been confirmed at the proteomic level. Additionally, most of these studies – including
276 those that examine the changes to the proteome – examine only one type of cancer and
277 compare that cancer only to HD samples. Furthermore, these analyses are heavily reliant
278 on cell lines and when patient samples are used, the number of patients included is very
279 small.

280 To gain a better understanding of the differences between hematological
281 malignancies, we have taken samples from patients suffering from three different B-cell
282 malignancies and utilized quantitative proteomics to identify protein signatures of each
283 malignancy as compared to both healthy B cells and the other disease types. With the
284 identification of over 8,000 proteins across all samples, this study provides an in-depth
285 analysis of the proteomes of each malignancy type and utilizes the findings in MCL to
286 validate the results. Overall, the study identifies proteins that are specifically dysregulated
287 in MCL that may prove to be therapeutic targets for MCL therapies in the future.

288 Given the ever-increasing prevalence of CAR-T therapy in the treatment of B-cell
289 malignancies, our study identified a significant number of surface proteins that may prove
290 to be novel CAR-T targets. We also found that patients may benefit from proteomic
291 analysis of proteasome subunits prior to Bortezomib-based treatments. One of the most
292 striking findings of this study is the differences between the proteomic signature and the
293 previously identified GEPs of these patients. Because most MCL patients exhibit a
294 heterogenous mixture of genomic mutations, it is difficult to treat the disease, and this
295 lends to the high mortality rate of these patients. We had expected to see that patients
296 who displayed alterations in genes frequently mutated in MCL such as ATM, TP53, and
297 UBR5 would cluster together and show similar changes in their proteome. However, we

298 found that the number and type of mutations present in these patients had little to no
299 effect on the proteins that are either up or downregulated. Furthermore, the finding that
300 only three of the 10 proteins specifically upregulated in MCL also exhibited increases in
301 mRNA reinforces the importance of examining the patient's proteome in addition to their
302 genome to identify therapeutic targets for treatment.

303 The presence of MARCKS and EHD1 in our study, two proteins whose
304 corresponding gene expression has previously been reported to be upregulated in MCL
305 as compared to CLL and in NHL as a whole, respectively, validates these findings.[11, 30] In
306 addition to MARCKS and EHD1, only one of the other 10 identified proteins had a
307 transcriptional change, DBN1. It has previously been identified that DBN1 is a direct target
308 of SOX11, a protein known to be aberrantly expressed in MCL.[31] The role that these three
309 proteins play in actin cytoskeleton formation, membrane formation, and the formation of
310 cellular projections suggest that they may be important for disease progression and
311 infiltration into secondary lymphoid tissues. Although it does not have a transcriptional
312 change present like the other three, identification of GSTP1 as overexpressed at the
313 proteomic level also validates the study. GSTP1 has been shown to be chronically
314 overexpressed in MCL and may provide resistance to drug therapies as its inhibition
315 increases chemotherapy sensitivity in MCL patients.[32] Because MCL patients chronically
316 relapse following current drug regimens, targeting GSTP1 in conjunction with these
317 therapies may prove to be an effective strategy to improve patient outcomes.

318 The other six proteins specifically upregulated in MCL are also transcriptionally
319 unchanged, indicating that they would have remained unidentified by typical patient
320 screening methods. These six proteins have never been associated with MCL previously.

321 STMN1 is known to be overexpressed in other types of leukemias and lymphomas.[33]
322 LGALS9 has been shown to be upregulated on the surface of obese B-ALL patients, and
323 targeting this protein was reported to promote cell death, disrupt cell cycle progression,
324 and prolong survival in B-ALL patients.[34] FHL1 stabilization leads to Ibrutinib resistance
325 in diffuse large B-cell lymphoma.[35] HMGB3, PDLIM1, and DDAH2 have never been
326 associated with any form of lymphoma previously, making them of particular interest to
327 this study.

328 In conclusion, this study highlights the importance of utilizing proteomics when
329 determining the treatment strategy for lymphoma patients. This data can be used as a
330 resource for identifying novel treatment strategies not only in MCL, but in other B-cell
331 malignancies. It identifies several new potential CAR-T therapy targets as well as novel
332 proteins of interest for drug discovery or PROTAC-based drug development, highlighting
333 the importance of the work.

334

335 **METHODS**

336 **Patient Samples**

337 The study was approved by the institutional review board of the University of Nebraska
338 Medical Center (UNMC) (203-15-EP) and performed in accordance with the Declaration
339 of Helsinki. Matched gene expression microarray data are available through the Gene
340 Expression Omnibus, accession GSE132929.[14, 29] Samples obtained from the UNMC
341 Lymphoma Tissue Bank were prioritized for inclusion in this study if they had previously
342 undergone pathology review, been interrogated by Affymetrix U133 Plus 2.0 gene
343 expression microarrays, and exome sequenced.[9,28] Patient samples are at diagnosis, and
344 annotated for gender, age at diagnosis, OS, and mutational profile (**Supplemental Table**

345 **S1**). Protein was isolated from 16 MCL, 7 FL, and 7 CLL patients. For the 5 HD patient
346 samples, either adult $CD19^+$ B cells were purchased from Stem Cell Technologies or
347 magnetic selected $B220^+$ B cells were isolated from tonsils. All samples were de-identified
348 and accompanied by the diagnosis from the medical records, plus overall survival and
349 patient mutations. Mutation and CNA data are publicly viewable through cBioPortal:
350 https://www.cbioportal.org/study/summary ?id=mbn_mdacc_2013.[9]
351

352 **Mass Spectrometry**

353 Solid tumors from MCL and FL were ground to powder in liquid nitrogen. Samples were
354 prepared for TMT labelling, as previously described.[13] Samples were prepared in five
355 separate batches each containing a combined sample control (4μg from each sample)
356 and a mixture of HD, CLL, FL, and MCL samples. Protein identification was performed
357 using proteome discoverer software version 2.4 (ThermoFisher, Waltham, MA) by
358 searching MS/MS data against the UniProt human protein database downloaded
359 February 2022. Data was normalized to the average abundance of the batch control for
360 each of the five batches. Analyses were performed on the ratio of the abundance of
361 normalized protein in batch control to individual patient sample. Data are available via
362 ProteomeXchange with identifier PXD038937.
363

364 **Flow Cytometry**

365 Cells were stained for 1 h on ice with indicated antibodies in 3% FBS in PBS. Data was
366 then analyzed using FlowJo software (Treestar, Ashland, OR). Antibody information is
367 available in supplementary materials and methods.
368

369 **Cell Culture**

370 Jeko-1, Mino, REH, and Ramos cells were purchased from ATCC (Manassa, VA) and
371 Granta519, HG-3, and MEC-1 cells were purchased from DSMZ (Leibniz Institute,
372 Germany). Cell lines were cultured following company specifications. All cells were
373 confirmed mycoplasma negative monthly while in culture.

374
375 **Western Blot**

376 Samples were prepared and analyzed as previously described.[13] Antibody information is
377 available in supplemental materials and methods.

378
379 **Statistical analysis**

380 All experiments were performed in triplicate unless noted. Proteins with a fold change ≥
381 1.5 were considered as upregulated, ≤ 0.5 as downregulated, and with a p-value ≤ 0.05
382 as significant. Statistical analyses were performed using unpaired two-tailed Student's t-
383 test assuming experimental samples of unequal variance (with Welch's correction). Error
384 bars depict the standard deviation ± the mean. PCA plots were generated using ClustVis
385 online tool (https://biit.cs.ut.ee/clustvis/). Heat maps were completed using Morpheus
386 online tool (https://software.broadinstitute.org/morpheus), using hierarchical clustering
387 and $\log_2$ transformation of protein abundance. Volcano plots were made using GraphPad
388 Prism v10.0.2 (GraphPad, La Jolla, CA). Gene ontology (GO) pathway analysis was
389 performed using DAVID Bioinformatics Database v6.8
390 (https://davidbioinformatics.nih.gov/home.jsp). Outputs were first sorted by count, and
391 then by p-value. All outputs with ≥ 5 associated genes and a p-value ≤ 0.05 are included
392 for reference in supplementary tables. Graphs were made using the top 5 hits for both
393 count and p-value. GO terms with similar names and gene were only listed once under

394 the first instance. For survival curve analyses, the p-value was calculated using a Log-
395 rank (Mantel-cox) test.
396

397 **AUTHOR CONTRIBUTIONS**

398 S.A.S., and S.M.B. conceived and designed the experiments; S.A.S., C.B.W., K.K.D.,
399 K.J.W., and S.M.B. performed experiments and analysis, H.C.H.L., and N.T.W. provided
400 technical and material support; J.M.V., T.G., and M.R.G. provided samples and/or clinical
401 data. S.A.S. and S.M.B. wrote the manuscript. All authors reviewed the manuscript before
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415

416 **FIGURE LEGENDS**

417 **Figure 1: Quantitative proteomics identifies protein landscapes of lymphoid**
418 **neoplasms as compared to healthy B cells**

419 **A.** Schematic of sample processing workflow of protein from either healthy donor (HD) B
420 cells or lymphoid tumors from three different B-cell malignancies: mantle cell lymphoma
421 (MCL), chronic lymphoblastic leukemia (CLL), and follicular lymphoma (FL). Combined
422 protein from all samples were processed the same as individual samples and used as a
423 control in each run. **B.** Filtering parameters of LC-MS/MS outputs from the five different
424 MS batches. 24,794 peptides corresponding to 8,027 proteins were filtered to only include
425 those with an abundance output (5,114). Analysis was performed on proteins identified in
426 all batches (1,502). **C.** PCA plot using ClustVis software of normalized abundances for all
427 samples. **D.** Heat map of the Log_2 value of normalized abundances for all samples.
428 Hierarchical clustering was performed using Euclidian distance for both rows and
429 columns. **E.** Venn Diagram of common upregulated (≥1.5-fold) (above) and
430 downregulated (≤0.5-fold) (below) proteins between MCL, FL, and CLL all as compared
431 to HD samples. **F.** GO terms for upregulated (≥1.5-fold) and downregulated (≤0.5-fold)
432 proteins for each cancer as compared to HD. **G.** Volcano plots of the Log_2 fold change
433 for each cancer as compared to HD showing significant ($p \leq 0.05$) upregulated (≥1.5-fold)
434 and downregulated (≤0.5-fold) proteins. Proteins upregulated in both MCL and FL
435 depicted in orange, proteins upregulated in MCL in purple, proteins upregulated in FL in
436 pink, proteins upregulated in CLL in light blue, and downregulated proteins of interest in
437 light green.

438

439 **Figure 2: Cell surfaceome identifies potential CAR-T cell therapy targets in MCL**

440 **A.** Pie chart of localization of 1,502 identified proteins highlighting 120 plasma membrane
441 proteins. **B.** Relative protein abundance of cell surface markers associated with B-
442 lymphoid malignancies (CD5, CD19, CD22). **C.** Heat map of plasma membrane proteins
443 that are differentially expressed in at least one of the three different B lymphoid
444 malignancies as compared to HD. **D.** Cell surface expression by flow cytometry in normal
445 human peripheral blood (PB), and B-Lymphoid derived cell lines Jeko1 (MCL), Granta519
446 (MCL), and HG3 (CLL). **E.** Relative abundance of CD81 and **F.** CD22 in CD19 high and
447 low expression patients. ns = not significant, * = p-value ≤ 0.05, ** = p-value ≤ 0.01.
448

449 **Figure 3: Analysis of proteasome components suggest precision targeted**
450 **therapeutics could improve patient outcomes**

451 **A.** PCA plot of MCL patient samples with clustering determined by ClustVis software. **B**.
452 Heatmap of proteasome subunits in all patients clustered by Pearson's Correlation.

453
454
455 **Figure 4: Identification of 10 post-transcriptionally regulated proteins specifically**
456 **upregulated in MCL**

457 **A.** Venn Diagram of proteins significantly ($p \leq 0.05$) upregulated (≥1.5-fold) (left) and
458 downregulated (≤0.5-fold) (right) in MCL samples as compared to HD, FL, and CLL
459 samples. **B.** Violin plots of relative protein abundance for 10 proteins specifically
460 upregulated in MCL as compared to HD, CLL, and FL. **C.** Violin plots of relative gene
461 expression as determined by RNA microarray of the 10 proteins from A in MCL as
462 compared to HD. **D.** Western blot confirmation in select MCL, CLL, and FL primary patient
463 samples and **E**. MCL, CLL, B-cell Acute Lymphoblastic Leukemia (B-ALL), and Burkitt's

464 Lymphoma (BL) patient derived cell lines as compared to HD. * = p-value ≤ 0.05, ** = p-
465 value ≤ 0.01, *** = p-value ≤ 0.001, **** = p-value ≤ 0.0001.

466

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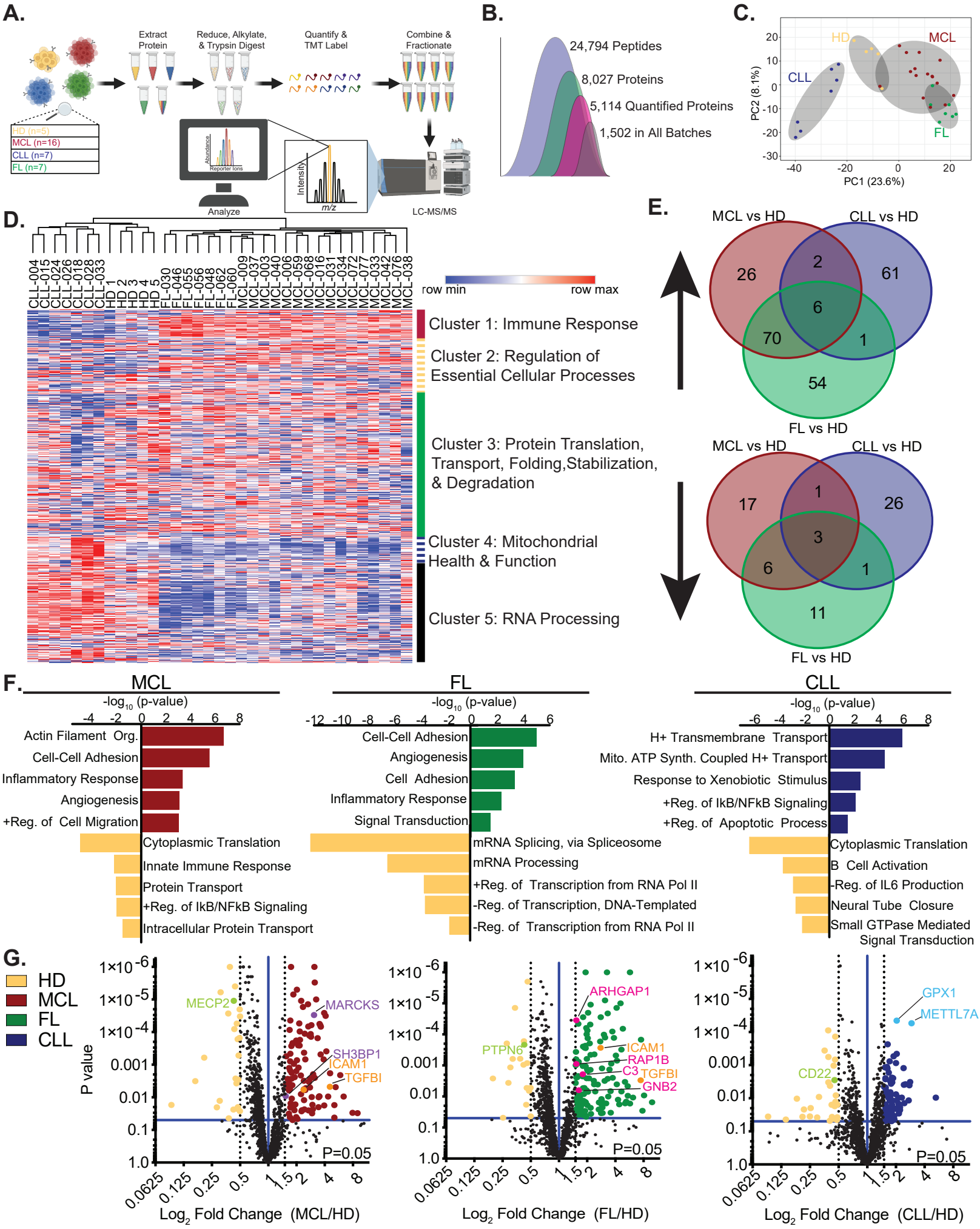
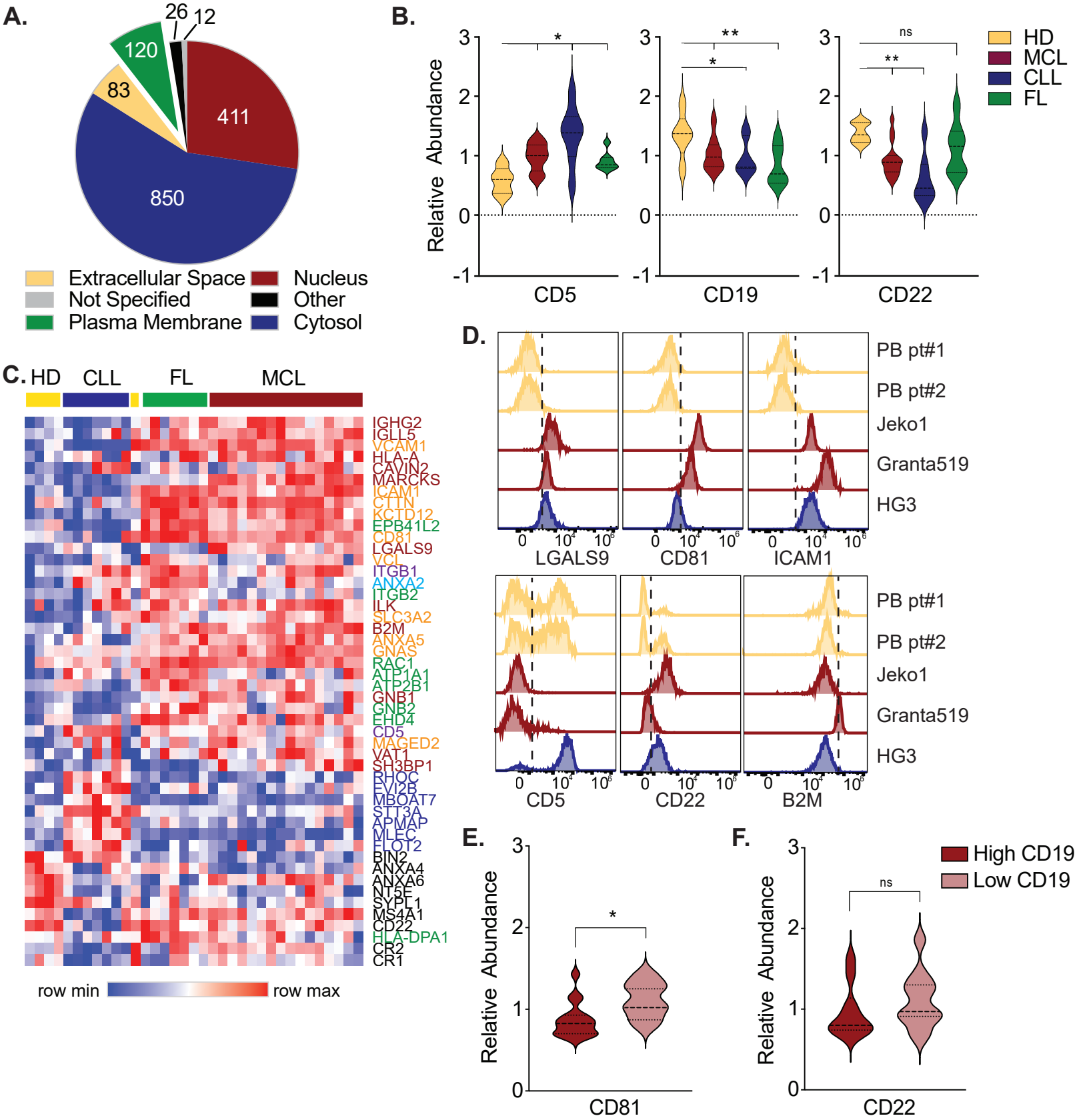
607

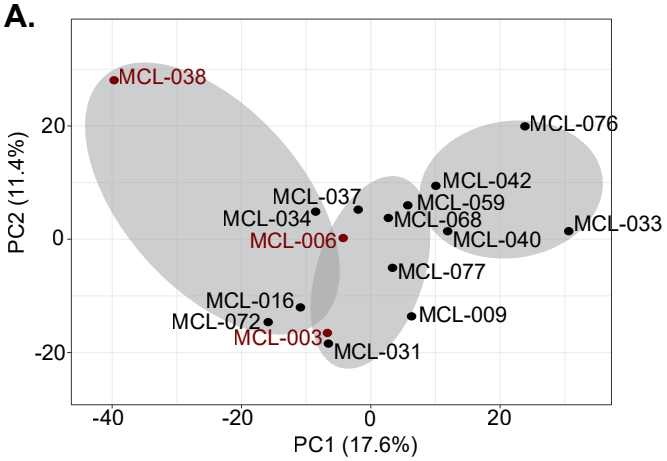
Swenson, et al.- Figure 1

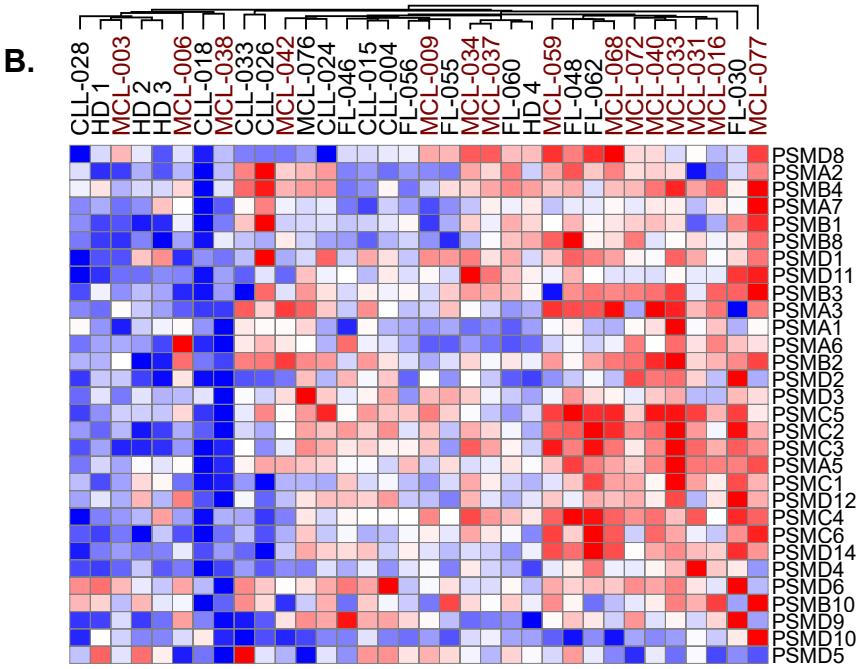
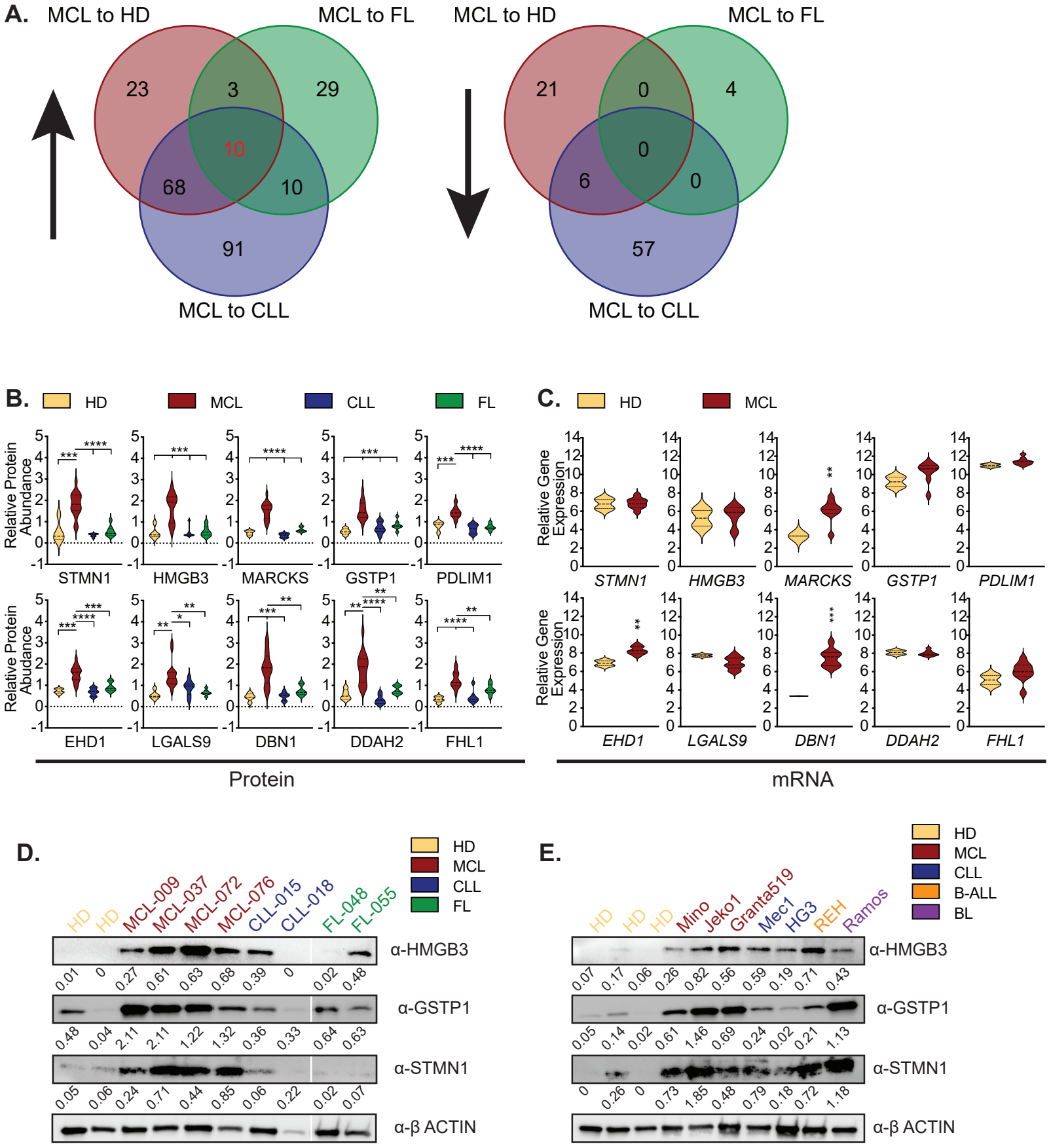


Swenson, et al. MCL Patients Figure 2



Swenson, et al. MCL Patients Figure 3





Swenson, et al. MCL Patients Figure 4