Platelet proteo-transcriptomic profiling validates mediators of thrombosis and proteostasis in patients with myeloproliferative neoplasms

Sarah Kelliher[1,2⊕,3,4], Sara Gamba[5], Luisa Weiss[4,6], Zhu Shen[2], Marina Marchetti[5], Francesca Schieppati[5], Caitriona Scaife[7], Stephen Madden[8], Kathleen Bennett[9], Anne Fortune[1,3], Su Maung[1,3], Michael Fay[1,3], Fionnuala Ní Áinle[1,3,4,11], Patricia Maguire[4,6,12], Anna Falanga[5,13], Barry Kevane[1,3,4#] and Anandi Krishnan[2,14,15#*]

Affiliations

[1]School of Medicine, University College Dublin, Dublin, Ireland

[2]Stanford University School of Medicine, Stanford University, Stanford, CA, USA

[3]Department of Haematology, Mater Misericordiae University Hospital, Dublin, Ireland

[4]UCD Conway SPHERE Research Group, University College Dublin, Dublin, Ireland

[5]Department of Immunohematology and Transfusion Medicine, Hospital Papa Giovanni XXIII, Bergamo, Italy

[6]School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland.

[7]UCD Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

[8]Data Science Centre, Royal College of Surgeons in Ireland, Dublin, Ireland

[9]School of Population Health, RCSI University of Medicine and Health Sciences, Dublin, Ireland

[10]Department of Haematology, Rotunda Hospital, Dublin, Ireland

[11]School of Medicine, Royal College of Surgeons in Ireland

[12]UCD Institute for Discovery, University College Dublin, Dublin, Ireland

[13]University of Milano-Bicocca, Department of Medicine and Surgery, Monza, Italy

[14]Rutgers University, Piscataway, NJ

[15]Stanford Cancer Institute, Stanford, CA, USA

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A.K. and B.K. are co-senior authors

*Corresponding Author:
Anandi Krishnan, PhD
Stanford University, Stanford, CA
anandi.krishnan@stanford.edu

Highlights

MPN patient platelet proteome identifies key pathobiological mediators of thrombosis and proteostasis.

The MPN platelet proteomic profile validates our prior findings from the platelet transcriptome.

45
46 **Abstract**

47 Patients with chronic Myeloproliferative Neoplasms (MPN) including polycythemia vera (PV) and essential

48 thrombocythemia (ET) exhibit unique clinical features, such as a tendency toward thrombosis and

49 hemorrhage, and risk of disease progression to secondary bone marrow fibrosis and/or acute leukemia.

50 Although an increase in blood cell lineage counts (quantitative features) contribute to these morbid sequelae,

51 the significant qualitative abnormalities of myeloid cells that contribute to vascular risk are not well understood.

52 Here, we address this critical knowledge gap via a comprehensive and untargeted profiling of the platelet

53 proteome in a large (n= 140) cohort of patients (from two independent sites) with an established diagnosis of

54 PV and ET (and complement prior work on the MPN platelet transcriptome from a third site). We discover

55 distinct MPN platelet protein expression and confirm key molecular impairments associated with proteostasis

56 and thrombosis mechanisms of potential relevance to MPN pathology. Specifically, we validate expression of

57 high-priority candidate markers from the platelet transcriptome at the platelet proteome (*e.g.,* calreticulin

58 (CALR), Fc gamma receptor (FcγRIIA*)* and galectin-1 (LGALS1*)* pointing to their likely significance in the

59 proinflammatory, prothrombotic and profibrotic phenotypes in patients with MPN. Together, our proteo-

60 transcriptomic study identifies the peripherally-derived platelet molecular profile as a potential window into

61 MPN pathophysiology and demonstrates the value of integrative multi-omic approaches in gaining a better

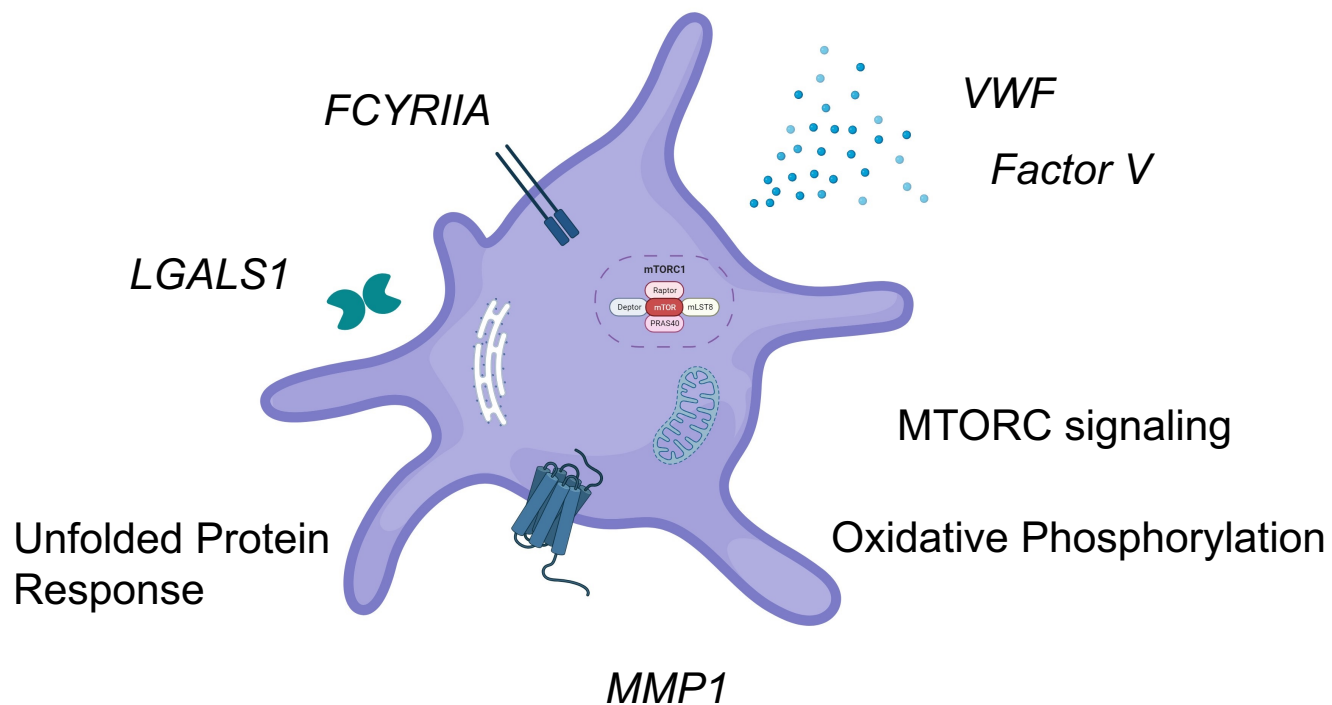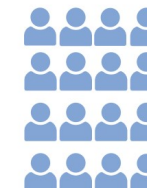62 understanding of the complex molecular dynamics of disease.
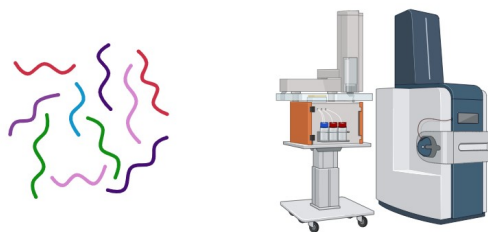
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Introduction

Myeloproliferative neoplasms (MPN) are chronic bone marrow malignancies characterised by clonal proliferation of hematopoietic precursors and elevated cell counts in peripheral blood. The three most common Philadelphia-chromosome negative (Ph⁻) MPN subtypes polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF), are diagnosed according to morphologic, clinical, laboratory, and genetic criteria[1,2]. Patients with MPN are at risk of progression to secondary bone marrow fibrosis or acute leukemia and experience a substantial burden of microvascular symptoms, negatively impacting their quality of life[3-5]. However, it is thrombosis, including both arterial and venous events, which represents the leading cause of morbidity and mortality for patients with PV and ET[6-9]. Several patient and disease-specific characteristics have been shown to correlate with increased clinical risk, yet despite currently available standard of care, the prediction and prevention of disease complications remains challenging[10].

The discovery of the *JAK2* V617F mutation in 2005 heralded a new era in the management of MPN, and the application of genomic techniques has radically modified diagnostic and prognostic approaches in the interim[11]. In addition to the MPN driver mutations (*JAK2* V617F, *CALR*, *MPL* & *JAK2* exon 12) there is increasing interest in other somatic gene mutations including epigenetic regulators, spliceosome components, transcription factors and oncogenes[12]. These parameters are key informants of risk stratification for patients with MF (MIPSS70[13]), yet clinical utility lags behind for the more indolent subtypes (PV & ET) where additional genomic information currently has limited impact on patient management[14]. Thus, greater understanding of fundamental MPN pathobiology, in particular, the molecular and cellular basis of disease, is urgently required to discover novel mediators that can be leveraged for prognostic and therapeutic purposes in these chronic MPN subtypes.

Platelets have a well-established and integral role in maintaining vascular integrity and mediating hemostasis. Additionally, platelets and their cargo have been shown in numerous translational studies to influence a myriad of pathological processes including inflammation, immune responses, and malignancy[15-17]. Platelet

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99   hyperactivity and the procoagulant role of platelets is a hallmark of MPNs[18] [19,20]. Yet, a comprehensive picture

100   of the MPN platelet molecular profile is still lacking. In recent published work that serves as a prelude to this

101   data[21], we evaluated a large (n=120) cohort of MPN patients of all three chronic subtypes, and discovered

102   progressive changes in platelet transcriptomic expression thus offering novel candidate signatures and

103   deeper insights into MPN pathobiology (*e.g.* interferon response, immuno-thrombosis, and impaired

104   proteostasis). However, to date, no studies have evaluated the unbiased platelet proteome in a sizeable

105   clinical cohort of patients with MPN and the overlap of MPN platelet proteo-transcriptome therefore, not

106   understood.

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108   In the present study, with the objective of delineating the MPN proteo-transcriptomic landscape, we performed

109   a comprehensive and untargeted quantitative profiling of the platelet proteome in a large (n= 140) cohort of

110   patients with an established diagnosis of PV and ET. We demonstrate marked differences in platelet protein

111   expression and discover novel MPN-altered pathways in platelets. Importantly, we validate, across two

112   independent sites, candidate signatures from the MPN platelet transcriptome at the protein level. Together,

113   this amounts to a comprehensive molecular profile of differential MPN pathobiology at high-sensitivity cellular

114   resolution.

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116   **Results**

117   **Platelet proteo-transcriptome across multi-site MPN clinical cohorts**

118   Patient samples were recruited for untargeted, quantitative proteomic analysis. Using established and

119   standardized platelet isolation protocols (see Methods), we prepared purified platelets from peripheral blood

120   samples of patients with an established diagnosis of MPN and a cohort of healthy controls recruited across

121   two sites: Hospital Papa Giovanni XXIII, Bergamo, Italy and Mater Misericordiae University Hospital, Dublin,

122   Ireland. Together, this platelet proteome compendium comprised of 140 human peripheral blood samples

123   from healthy donors (n= 40) and World Health Organization-defined MPN patients (n= 59 ET, n= 41 PV).

124   Pertinent clinical features are listed in **Table 1A** and shown in **Figure 1**; including MPN subtype (**A**), somatic

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125   driver mutation status (**B**), sex (**C**), treatment (**D**), age (**E**), and platelet count (**F**). Any inter-patient variability

126   in patient age, sex, and treatment as well as experimental batch effects were adjusted as confounding factors

127   in all downstream expression analyses (see Methods).

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129   For comparative proteo-transcriptomic analysis, we leveraged our published dataset of the MPN platelet

130   transcriptome comprising of 120 human peripheral blood samples from healthy donors (n= 21) and World

131   Health Organization–defined MPN patients (n= 99) (single center, Stanford University, CA). Clinical variables

132   of patients from this independent cohort (PV n= 33; ET n= 24, control n= 21) are outlined in **Table 1B** and

133   described extensively in the original publication[21].

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135   **MPN platelet proteome distinguishes disease phenotype and reveals prothrombotic, proinflammatory,**

136   **and profibrotic signatures**

137   Focusing for this study on the more prothrombotic subtypes of MPNs (PV and ET), we hypothesized that the

138   platelet proteome differs in MPN subtypes and would offer insights into the underlying pathophysiology and

139   the associated thrombotic phenotype. We compared platelet proteomic expression in PV and ET with that of

140   healthy donors and discovered distinct signatures. Unsupervised principal component analysis (PCA) of MPN

141   patients and controls (**Figure 2A**) confirmed that the collective variability from the first two principal

142   components was MPN disease status (26% of total variance, after adjusting for age, sex, treatment, and

143   experimental batch).

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145   A total of 1,952 platelet proteins were quantified across MPN and control samples. Differential expression

146   (DE) analysis of proteomic data (volcano plot**, Figure 2B, C**) efficiently distinguished each of the MPN

147   subtypes and resulted in highly significant expression signatures (false discovery rate FDR <0.05) with 227

148   proteins differentially regulated in ET (113 increased and 114 decreased), and 166 in PV (122 increased and

149   44 decreased) compared to healthy donors.

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151   Differential markers in ET and PV highlight candidate proteins as potential mediators of the clinically

152   encountered pro-thrombotic phenotype, including proteins associated with hypercoagulation (e.g. MMP1,

153   SERPINH1, FcγRIIA, PDIA6) and drivers of inflammation (LGALS1, S100A6, SLC25A24, CD63). Select

154   candidates are highlighted in **Figure 2B, C** and discussed in detail in **Table 2**. Also, while the profibrotic

155   phenotype of myelofibrosis is well-understood[22], candidate markers (LGALS1, S100A6) in the early, more

156   indolent subtypes of these chronic myeloid malignancies point to potential platelet mediators of fibrosis of

157   relevance to MF.

158   **Differential platelet proteome in MPNs identifies potential mediators of disease phenotype.**

159   To better decipher the functional and biological significance of the observed proteomic changes, we

160   performed pathway-enrichment analysis and identified signaling pathways that are differentially activated

161   between MPN subtypes and controls (**Figure 3**). Gene set enrichment analysis (GSEA, see Methods) found

162   that MPN (stratified by subtypes; ET and PV) primarily induces pathways associated with the unfolded protein

163   response. Impaired proteostasis is attributed[23,24] to endoplasmic reticulum stress (note overexpression of

164   candidate proteins HYOU, PDIA6, HSP90B1 in this data, **Figure 2B**). Moreover, among the most enriched

165   gene sets, MPN pathology induces activation of oxidative phosphorylation (OXPHOS) and mTORC1 signaling

166   pathways. Proliferation pathways also reveal significant enrichment with overexpression of c-MYC target

167   proteins among patients with PV. Representative GSEA profiles are shown in **Figure 3** and the full list of gene

168   sets are detailed in **Table S2A-B**. The MPN pathways exhibiting significant proteomic regulation by GSEA

169   are consistent with our observations at the individual level for increased or decreased protein expression and

170   mirror key signaling pathways identified in our prior platelet transcriptomic analysis[21]. These results are also

171   consistent with independent studies in MPN granulocytes[25,26] and hematopoietic stem and progenitor cells[27-

172   29] confirming their significance in MPN pathobiology and as potential therapeutic modalities[30].

173   **Shared platelet proteo-transcriptome by MPN subtype.**

174   Having defined differential protein signatures by MPN subtype, we proceeded to identify significant (FDR <

175   0.05) shared signatures between the current platelet proteomic data and our prior transcriptomic data[21]. We

176 found 214 differentially expressed markers (significant ones alone across MPNs) at both transcript and protein

177 levels – together translating to a moderate correlation (Spearman's rho =0.44) between each other (**Figure**

178 **4**). In general, this is consistent with previous transcriptome-proteome comparative studies, including across

179 species and matched cell-line experiments[31-33].

180 Taking the overlap of the significantly differentially expressed proteins and genes (FDR < 0.05) that were

181 common across MPN subtypes (i.e., present in both ET and PV specimens), we identified highly expressed

182 features (n= 17) shared between the two independent MPN 'omic' cohorts.   Unsupervised hierarchical

183 clustering further classified this core MPN platelet proteo-transcriptomic overlap for ET and PV subtypes, in

184 contrast with that of healthy donors. **Figure 5** demonstrates graded expression of 17 shared signatures

185 between the MPN platelet proteome and transcriptome. Although MPN patients cluster into a group distinct

186 from controls, we also note the overlay between PV and ET platelet signatures likely reflecting shared

187 pathobiology. Most importantly, these signatures point to key molecular pathways previously identified in our

188 MPN platelet transcriptome data and now validated at the protein level in the ET/PV platelet proteome

189 (Figures 2, 3, and 5). These include, for instance impaired proteostasis and ER stress (known markers[34-36]

190 PSMD13, UNC45A, ATP6V1B2, NUCB1), oxidative stress and apoptosis[37-40] (MAOB, PSAP, VDAC1, TIMP1)

191 and lipid metabolism[41-43] (ME1, DHCR7, FAH). Moreover, as shown earlier in **Figure 2B,** known markers of

192 platelet activation, classical coagulation factors and mediators of immunothrombosis such as FcγRIIA, F5,

193 VWF LGALS1, and LGALS3BP, are also confirmed.

194 Thus together, this comprehensive proteo-transcriptomic analysis validating MPN platelet signatures not only

195 between RNA and protein expression but also across two independent sites and clinical cohorts highlights

196 additional underlying mechanisms beyond the MPN genetic landscape (and the known pivotal driver and non-

197 driver mutations). The observed platelet molecular profile supports our hypothesis that platelets contribute to

198 remodeling of the circulatory microenvironment which could lead to a self-reinforcing inflammatory milieu,

199 possibly promoting disease progression and associated MPN vascular complications.

Discussion

Here, we present a comprehensive proteo-transcriptomic study of blood platelet expression in patients with chronic progressive MPNs across international multi-site clinical cohorts (first of this kind to the best of our knowledge). Thrombocytosis is a cardinal feature of MPN, however platelet count in isolation is not predictive of clinical outcome[44]. Moreover, conventional anti-platelet therapy does not fully mitigate thrombotic risk and cytoreductive therapy has shown limited potential to alter natural history of the disease to date[45]. Recent data suggest[46] that disease course may be modified with targeted treatments, however options remain limited, with incomplete understanding of why patients fail first line therapy with few available alternatives. One approach that is increasingly recognized as highly valuable in identifying novel therapeutic targets as well as molecular markers of disease (especially at a systems level) is to leverage pertinent multi-omic[47-49] datasets.

In this study, we unravel the molecular phenotype of platelets, with a focus on the platelet proteome in the highly prothrombotic subtypes of MPN (PV and ET). Crucially, we validate this proteomic data with our prior MPN platelet transcriptomic data (ET, PV, and MF), confirming a strong possible role for platelet mechanisms in MPN pathobiology. Our data also expands on findings from other smaller or targeted studies, thus identifying the platelet proteo-transcriptome as potential mediators (and therefore, an invaluable biosource) of MPN proinflammatory, prothrombotic, and profibrotic processes[50-56].

Our international collaborative effort demonstrates an example of addressing pertinent, global research questions (particularly for rare disorders such as MPNs). Importantly, this cross-institutional body of work emphasizes the value of standardized protocols in achieving larger cohort sizes and ensuring external validation toward high-quality and reproducible data of direct relevance to patient care[57,58].

In conclusion, using label-free, untargeted platelet proteomic profiling, we discover key pathobiological mediators of thrombosis and proteostasis in MPN patients (focusing on ET and PV subtypes) from multiple recruitment sites; and validate the proteome with our prior transcriptomic data from an independent cohort.

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226 We identify significant differential protein expression, that were also noted at the RNA level, thus

227 strengthening biological insights, and revealing high priority candidate markers for functional evaluation in

228 MPN studies. Our findings highlight the platelet molecular profile as a unique peripherally accessible window

229 into MPN pathobiology. We also demonstrate the immense value of collaboration between groups with shared

230 research goals and complementary expertise in increasing the feasibility of large-scale translational studies,

231 particularly for rare disease cohorts.

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233 **Limitations of the study**

234 There are several potential limitations of this study. Firstly, the comparative multi-omic measurements were

235 not in the same patients, but in separate MPN clinical cohorts at independent sites. Consequently, there were

236 limitations on the extent of integrative multi-omic analyses possible. However, our results do align with a

237 recent independent but smaller study with paired MPN platelet multi-omic analysis[50]. Of note, there is

238 increasing acknowledgement that a 'rectangular approach' using untargeted, discovery methodologies from

239 large, geographically independent cohorts of patients at both the discovery and validation phases of biomarker

240 research is superior to traditional 'triangular workflow', where hypothesis-free technologies are used initially

241 followed by targeted validation of a smaller candidate markers in a larger sample size[59]. Secondly, in line

242 with our hypothesis focusing on platelets as prothrombotic mediators we have limited our investigation to PV

243 and ET patient samples. However, our analysis revealed the heterogeneity of the platelet proteome and

244 implicated platelets in a diverse range of biological pathways (and confirming signatures identified in our prior

245 MPN platelet transcriptomic data). We recognize that a longitudinal study following patients for the

246 development of thrombotic outcomes (at adequate power) would be critical and will enable the development

247 of future risk prediction algorithms. Thirdly, data-dependent acquisition mass spectrometry was carried out in

248 this study, wherein a subset of the most abundant ions reaching the mass spectrometer detector are

249 individually fragmented. Incomplete protein coverage is a well described limitation of this technique,

250 particularly for low abundant proteins, and we recognize that failure to detect RNA-protein correlates in our

251 platelet samples does not suggest absence at the protein level. Finally, we recognize that future mechanistic

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252 studies are needed to substantiate results from this study focused on characterizing the MPN platelet proteo-

253 transcriptome alone. Our ongoing efforts include proteomic analysis of platelets from prospectively recruited

254 patients at the time of initial MPN diagnosis to identify candidates for mechanistic interrogation in treatment

255 naive patients and to better understand the platelet molecular signature as a potential diagnostic, prognostic,

256 or therapeutic target for patients with MPN.

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258 **Methods**

259 **Study recruitment and sample preparation for platelet proteomics**

260 Ethical approval was granted from the Institutional Review Board (IRB) of the Mater Misericordiae University

261 Hospital, Dublin, Ireland (IRB approval number 1/378/2241) and the Papa Giovanni XXIII Hospital, Bergamo,

262 Italy (IRB approval number 1789/2013). Patients over the age of 18 with an established diagnosis of MPN

263 (PV n= 41, ET n= 59) according to the World Health Organization diagnostic classification and a control group

264 of healthy donors (n= 40) were invited to participate (2014-2022)[60]. Following informed consent, samples of

265 whole blood collected in sodium citrate (0.105mol/L) were obtained by direct venipuncture. Platelets were

266 isolated from platelet rich plasma (PRP) obtained by centrifugation of whole blood for 10 minutes at 400 g

267 at room temperature (RT), according to a published method[61]. Briefly, PRP was diluted in 1:2 ratio

268 with Krebs Ringer buffer (4mM KCl, 107 mM NaCl, 20 mM $NaHCo_3$, 2mM Na_2SO_4, pH 5). After centrifugation

269 at 1,000 g for 10 min at RT, the platelet pellet was resuspended in Krebs Ringer buffer supplemented with

270 glucose (0.9 g/L, PH 6) and centrifuged a second time (1,000 g, 10 min, RT). This washing procedure was

271 repeated twice, and the platelets were resuspended at a concentration of 1x 10^9 platelets/mL in phosphate

272 buffered saline (PBS) or PBS containing 1% Triton , snap frozen on dry ice and stored at -80 °C.

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274 **Mass Spectrometry**

275 Whole platelets were lysed in RIPA buffer (100 mM Tris pH 8.0, 300 mM NaCl, 2% Triton-X 100, 0.2% SDS,

276 1% sodium deoxycholate) with protease and phosphatase inhibitors (Roche). Samples were precipitated with

277 95% acetone overnight at -20 °C, centrifuged at 14,000 g at 4 °C for 10 minutes and the supernatant was

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278 removed. The protein pellet was resuspended in PBS and protein concentration was estimated by measuring

279 absorbance at 280nm using a DS-11 spectrophotometer (DeNovix) as before[62,63]. Mass spectrometry sample

280 preparation was performed using the commercially available PreOmics iST HT 192x kit (P.O.00067). In brief,

281 50 µg of protein was simultaneously lysed, reduced, and alkylated for 10 min at 95 °C and 1000 rpm,

282 transferred to a cartridge and subsequently double-digested with LysC and trypsin at 37 °C and 500 rpm for

283 1 hour. Peptides were purified with repeated washes and eluted. Samples were evaporated at 45 °C and

284 peptides resuspended in LC-load buffer. Digested peptides were loaded onto C18 trap columns (Evotip) and

285 washed with 20 µL 0.1% formic acid (FA) followed by the addition of 100 µL storage solvent (0.1% FA).

286 Differential proteomic signatures were established using label free liquid chromatography mass spectrometry

287 (LC-MS) analyzed in a Bruker TimsTOF mass spectrometer connected to an EvoSep liquid chromatography

288 system operated by the UCD Conway Proteomics Core facility.

289 Samples were loaded onto the Evosep One LC system and separated with an increasing acetonitrile gradient

290 over 40 minutes at a flow rate of 250 nl/min at room temperature. The mass spectrometer was operated in

291 positive ion mode with a capillary voltage of 1500V, dry gas flow of 3 l/min and a dry temperature of 180 °C.

292 All data was acquired with the instrument operating in trapped ion mobility spectrometry (TIMS) mode.

293 Trapped ions were selected for MS/MS using parallel accumulation serial fragmentation (PASEF).

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295 Identified peptides from platelet samples were searched against a human FASTA (July, 2022) using

296 MaxQuant (2.0.3.0) with specific parameters for trapped ion mobility spectra data dependent acquisition

297 (TIMS DDA). In the main Andromeda search precursor, mass and fragment mass had an initial mass

298 tolerance of 6 ppm and 20 ppm, respectively. The search included fixed modification of carbamidomethyl

299 cysteine. Minimal peptide length was set to seven amino acids, and a maximum of two miscleavages was

300 allowed. The false discovery rate (FDR) was set to 0.01 for peptide and protein identifications. The normalized

301 protein intensity of each identified protein was used for label free quantitation (LFQ) as previously described[64].

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Proteomic analyses

Statistical analysis of the LFQ intensities was performed using Perseus (version2.0.10) and R (version 4.3.1). Protein identifications were filtered to eliminate identifications from the reverse database, proteins only identified by site, and common contaminants. For downstream analysis, only proteins identified in at least 50% of samples in at least one group (control/ET/PV) were included. Missing values were imputed using the random forest method (Missforest package, R/Bioconductor). Data was log2-transformed and differential protein expression was established using the Limma software package within R/Bioconductor.

Differential protein expression was adjusted for batch, patient age, sex, and treatment (antiplatelet and cytoreductive therapy) as potential confounding variables within the linear model in Limma (design <- model.matrix(~patientvar$Subtype+patientvar$Batch+patientvar$Age+patientvar$Sex+patientvar$ASAnum +patientvar$HYDnum). Controlling for multiple comparisons was performed using the Benjamini-Hochberg defined false discovery rate (FDR). Significant differential protein expression was pre-specified as proteins with an FDR < 0.05 and a fold change of 1.5 in MPN, as compared to healthy controls.

Continuous data were summarized as medians and IQRs and categorical data are presented as frequencies and percentages. To compare differences in clinical variables between healthy controls and MPN subtypes (ET and PV), we used violin and box plots and conducted Mann-Whitney U test for non-parametric data. For unsupervised clustering and visualization, we performed principal component analyses (identifying MPN subtypes by color). All analyses were performed using the R studio interface (version 2023.03.1+446).

Proteomic quality control and validation analysis

To assess intra-donor platelet proteomic reproducibility, 6 patient samples were analyzed as technical replicates (5 in duplicate, 1 in triplicate). Pearson correlation coefficient (r) was performed on the log2 transformed LFQ- intensity of all proteins quantified (n= 1771) across technical replicate samples. To assess biological (inter-donor) variability in protein abundances, Pearson correlation coefficient (r) was performed on

329   the log2 transformed LFQ-intensity of all proteins quantified (n= 1952) across biologic replicate samples

330   (control n= 40; MPN n= 100).

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332   **Pathway/Gene set enrichment analysis for differentially expressed (DE) proteins.**

333   Gene set enrichment analysis(GSEA)[65], a well-established method for determining regulatory patterns in co-

334   expressed genes, was performed on the entire DE protein set for each MPN subtype (PV & ET), using the

335   Cancer Hallmarks gene sets from MSigDB[66]. The 'GSEA Pre-ranked' function was used with a metric score

336   that combines fold change and adjusted p-value together for improved gene ranking. We used default settings

337   with 10,000 gene set permutations to generate p and q values and compared MPN subtypes. In these

338   analyses, to allow for a broad comparison, we assessed all transcripts that were differentially expressed

339   according to FDR/adjusted p < 0.25 as recommended by the authors of GSEA [65].

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341   **Transcriptomic analyses**

342   For platelet transcriptomics, detailed methodology including platelet isolation, next generation RNA

343   sequencing and platelet transcriptome analysis is outlined in the original publication[21]. Briefly, peripheral blood

344   samples from 99 patients with MPN (ET n= 24, PV n= 33, MF n= 42) were obtained from the Stanford Cancer

345   Institute Hematology Tissue Bank from December 2016- December 2019 and 21 healthy donors from the

346   Stanford Blood Center. All blood samples were obtained with written informed patient or donor consent.

347   Ethical approval was granted by the Stanford University Institutional Review Board (IRB approval #18329).

348   RNA Sequencing was performed at the Stanford Genomics core. Platelet transcriptomic data were library-

349   size-corrected, variance-stabilized, and log2-transformed using the R package DESeq2[67].

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351   **Comparative proteo-transcriptome analysis**

352   Our published MPN platelet RNA sequencing dataset was used for comparative analysis with the MPN mass

353   spectrometry proteomic data. Comparative analysis of these two independent 'omic' cohorts was established

354   using direct feature overlap of significantly expressed genes and proteins (FDR <0.05). Shared candidate

355   markers between the two independent MPN -omic cohorts were evaluated for further analyses. We generated

356   heatmaps of these common highly significant genes and proteins using the pheatmap R package and its built-

357   in functions for hierarchical cluster analysis on the sample-to-sample Euclidean distance matrix of the

358   expression data for each independent dataset. Spearman's rank correlation coefficient (rho) was used to

359   correlate differential gene expression (log2fold change) of common significant (FDR < 0.05) genes and

360   proteins between the independent MPN platelet proteomic and transcriptomic cohorts.

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407 **Authorship Contributions** S. Kelliher, B. Kevane, and A. Krishnan conceived of the overall study and

408 secured funding. S.K. designed the experimental plan with input from P.M., F.N.A., B.K. and A.K. S.K. and

409 S.G. coordinated and performed the sample acquisition and processing according to established protocols.

410 Z.S. and A.K. developed the code for computational analyses and A.K. and S.K. performed and interpreted

411 the analyses. S. Madden and K.B. provided bioinformatic support. L.W. supported platelet protein preparation

412 and C.S. operated mass spectrometry at the UCD Conway Proteomics Core Facility. Clinical samples, and

413 annotation were kindly provided by A. Falanga and M.M., F.S. and S.G. at the Hospital Papa Giovanni XXIII,

414 Bergamo, Italy. A. Fortune., S. Maung, M.F. provided clinical samples and annotation at the Mater

415 Misericordiae University Hospital, Dublin, Ireland. S.K., and A.K. wrote and edited the manuscript. All authors

416 critically reviewed and edited the manuscript. All authors approved the final manuscript.

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418 **Data Sharing Statement**

419 The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the

420 PRIDE[68] partner repository with the dataset identifier PXD046324. RNA-sequencing data from this work

421 (original FASQ files from paired-end sequencing of all 120 samples) is already deposited to the NIH genomic

422 data repository dbGAP under public accession # PHS-0021-21. v1.P1.

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427 **Table 1A: Characteristics of Patients & Controls Included in the Proteomic Cohort**

	ET	PV	Control
Subject Count, n	59	41	40
Sample Count, n	59	41	40
Median age, y (range)	61 (32-86)	63 (38-83)	45 (24-61)
Female, n (%)	32 (54)	22 (54)	24 (60)
Platelet count, median (25% quartile, 75% quartile)	540 (422, 778)	465 (318, 638)	244 (220, 265)
MPN driver mutation, n (%)			
JAK2 V617F	26 (44)	41 (100)	0 (0)
CALR	18 (31)	0 (0)	0 (0)
MPL	2 (3)	0 (0)	0 (0)
Triple Negative	12 (21)	0 (0)	0 (0)
Missing	1 (1)	0 (0)	0 (0)
Therapy, n (%)			
Aspirin & hydroxyurea	27 (46)	28 (68)	0 (0)
Aspirin only	20 (34)	12 (30)	0 (0)
Hydroxyurea only	4 (7)	0 (0)	0 (0)
JAK inhibitor	0 (0)	0 (0)	0 (0)
No treatment	8 (13)	1 (2)	40 (100)

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456 **Table 1B: Characteristics of Patients & Controls Included in the Transcriptomic Cohort**
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	ET	PV	Control
Subject Count, n	24	31	21
Sample Count, n	24	33	21
Median age, y (range)	58 (30-78)	60 (27-84)	57 (26-69)
Female, n (%)	16 (66)	14 (45)	5 (24)
Platelet count, median (25% quartile, 75% quartile)	562 (464,799)	447 (310, 557)	167 (146,185)
MPN driver mutation, n (%)			
JAK2 V617F	13 (54)	31 (100)	0 (0)
CALR	7 (30)	0 (0)	0 (0)
MPL	2 (8)	0 (0)	0 (0)
Triple Negative	2 (8)	0 (0)	0 (0)
Missing	0 (0)	0 (0)	0 (0)
Therapy, n (%) **Aspirin& hydroxyurea**			
Aspirin only	6 (25)	10 (32)	0 (0)
Hydroxyurea only	10 (42)	12 (39)	0 (0)
JAK inhibitor	5 (21)	1 (3)	0 (0)
No treatment	1 (4)	7 (23)	0 (0)
	2 (8)	3 (9)	21 (100)

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469   **Table 2**

Candidate protein (Gene name)	Platelet differential expression by MPN subtype Absolute fold change (direction of change)	Cellular function and relationship to procoagulant, proinflammatory, and profibrotic pathways in other published work.
MMP1	**PV:** 4.5 (increased) **ET:** 5.2 (increased)	MMP1, an interstitial collagenase, is known to cleave protease-activated receptor 1 (PAR1) and promote platelet activation and regulate thrombogenesis in vitro[69-71]. Furthermore, MMP1 mediates tumor invasion by compromising vascular barrier integrity and has been associated with inferior prognosis in solid organ malignancies[72-74].
FcγRIIA	**PV:** 2 (increased) **ET:** 2 (increased)	Unbalanced FcγRIIA-mediated platelet aggregation was previously reported to promote thrombosis[75].
SERPINH1	**PV:** 2 (increased)	There is evidence of decreased expression or ablation of this collagen binding, platelet adhesion protein in immobilized mammals as a thromboprotective mechanism[76-78].
LGALS1	**PV:** 6.7 (increased) **ET:** 4.7 (increased)	Galactin-1 is a beta-galactosidase binding protein which is reported to promote tumour cell proliferation and survival in haematological malignancies[79]. Recent data delineates the contribution of galactin-1 to disease severity in myelofibrosis and identified the protein as a potential drug target with disease modifying effects[80].
S100A6	**ET:** 2 (increased)	The S100 family of proteins is a major player in hematopoietic proliferation and recent work has identified proinflammatory/profibrotic roles for S100A6, S100A8, S100A9 in bone marrow, granulocytes, and plasma in MPN[80-85].
PDIA6	**PV:** 1.7 (increased)	Protein disulfide isomerases (PDIs) are key mediators of platelet ER homeostasis and the relationship between PDIs, ER & oxidative stress, platelet activation and thrombosis has recently been elucidated[86,87].
HPSE	**PV:** 1.8 (increased)	Heparanase cleaves heparan sulfate proteoglycans and participates in extracellular matrix remodeling. It has been shown to be increased in the plasma extracellular vesicles of patients with PV[56].
DIAPH1	**PV:** 1.9 (increased) **ET:** 1.8 (increased)	We find increased expression of protein diaphanous homolog-1 (DIAPH1) possibly suggesting altered megakaryopoiesis in peripherally circulating platelets in PV and ET. DIAPH regulates proplatelet formation via Rho-mediated actin polymerization and microtubule assembly[88].
RAB4A	**PV:** 3.7 (increased) **ET:** 2.3 (increased)	A Ras GTPase signaling protein which regulates platelet alpha granule release[89].

CD63	**PV: 2.6** (decreased)	Downregulation of CD63 has been associated with proliferation and metastasis in solid organ malignancy regulated by IL-6, IL-27 and STAT3 signaling[90].
CTSC	**PV:** 2 (increased)	Abundant Cathepsin C drives inflammation through macrophage activation via NF-κB signaling pathway[91].
VAMP8	**ET:** 1.7 (increased)	VAMP8 regulates platelet granule secretion and thrombosis *in vivo*[92].
EIF4G1	**PV:** 2.1 (increased) **ET:** 1.74 (increased)	EIF4G1 has been identified as a prognostic biomarker in breast cancer[93].
HSP90AB1	**ET:** 1.5 (increased)	We find increased expression of heat shock proteins in ET (HSP90AB1, TRAP1) and PV (HYOU1, HSPH1, DNAJA2). Heat shock protein 70 kDa and heat shock protein 90 kDa are two families of chaperone networks with integral roles in protein folding, degradation, trafficking, and maturation. They are known to promote oncogenesis by protecting a spectrum of cancer related proteins[94-96].
SLC25A2	**PV:** 1.9 (decreased)	SLC25A2 is decreased in PV (with SLC2A3 and SLC44A1 differentially expressed in ET). Solute membrane carrier proteins have been associated with venous thromboembolism in genome wide association studies and *in vivo* models[97,98].
RAB32	**PV:** 2.1 (increased) **ET:** 1.6 (increased)	RAB32 is increased in ET (along with mitochondrial membrane protein TOMM22). Mitochondria are recognized as key regulators of platelet procoagulant function[99]. Loss of mitochondrial protein RAB32 is associated with dense granule storage pool disease Hermansky-Pudlak syndrome[100].
PSMD11	**PV:** 2 (increased) **ET:** 1.7 (increased)	We show evidence of dysregulated protein degradation pathways with upregulation of PSMD11 along with differential expression of lysosomal proteins (SORT1 and ATP6V) and other proteasomal subunits. This reflects the work of other groups who have shown that protein quality-control pathways may be important in the pathogenesis of MPN and other prothrombotic diseases and represent novel therapeutic targets[101-103].

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476   Bibliography
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478 1. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of
479   Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. Jul
480   2022;36(7):1703-1719. doi:10.1038/s41375-022-01613-1

481 2. Gianelli U, Thiele J, Orazi A, et al. International Consensus Classification of myeloid and lymphoid
482   neoplasms: myeloproliferative neoplasms. *Virchows Arch*. Jan 2023;482(1):53-68. doi:10.1007/s00428-
483   022-03480-8

484 3. Hultcrantz M, Björkholm M, Dickman PW, et al. Risk for Arterial and Venous Thrombosis in Patients
485   With Myeloproliferative Neoplasms: A Population-Based Cohort Study. *Ann Intern Med*. Mar 6
486   2018;168(5):317-325. doi:10.7326/m17-0028

487 4. Baumeister J, Chatain N, Sofias AM, Lammers T, Koschmieder S. Progression of Myeloproliferative
488   Neoplasms (MPN): Diagnostic and Therapeutic Perspectives. *Cells*. Dec 16
489   2021;10(12)doi:10.3390/cells10123551

490 5. Shi D, Shi H, Liu X, et al. Variables associated with patient-reported outcomes in patients with
491   myeloproliferative neoplasms. *Leuk Lymphoma*. Nov 2021;62(11):2703-2715.
492   doi:10.1080/10428194.2021.1933481

493 6. Hamulyák EN, Daams JG, Leebeek FWG, et al. A systematic review of antithrombotic treatment of
494   venous thromboembolism in patients with myeloproliferative neoplasms. *Blood Adv*. Jan 12
495   2021;5(1):113-121. doi:10.1182/bloodadvances.2020003628

496 7. Barbui T, De Stefano V, Falanga A, et al. Addressing and proposing solutions for unmet clinical needs
497   in the management of myeloproliferative neoplasm-associated thrombosis: A consensus-based position
498   paper. *Blood Cancer J*. Aug 8 2019;9(8):61. doi:10.1038/s41408-019-0225-5

499 8. Falanga A, Marchetti M. Cancer-associated thrombosis: enhanced awareness and pathophysiologic
500   complexity. *J Thromb Haemost*. Jun 2023;21(6):1397-1408. doi:10.1016/j.jtha.2023.02.029

501 9. Falanga A, Marchetti M. Thrombotic disease in the myeloproliferative neoplasms. *Hematology Am Soc
502   Hematol Educ Program*. 2012;2012:571-81. doi:10.1182/asheducation-2012.1.571

503 10. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2019 update on diagnosis, risk-
504   stratification and management. *Am J Hematol*. Jan 2019;94(1):133-143. doi:10.1002/ajh.25303

505 11. Vannucchi AM, Guglielmelli P. Molecular prognostication in Ph-negative MPNs in 2022. *Hematology
506   Am Soc Hematol Educ Program*. Dec 9 2022;2022(1):225-234. doi:10.1182/hematology.2022000339

507 12. Marneth AE, Mullally A. The Molecular Genetics of Myeloproliferative Neoplasms. *Cold Spring Harb
508   Perspect Med*. Feb 3 2020;10(2)doi:10.1101/cshperspect.a034876

509   13. Tefferi A, Guglielmelli P, Lasho TL, et al. MIPSS70+ Version 2.0: Mutation and Karyotype-Enhanced
510   International Prognostic Scoring System for Primary Myelofibrosis. *J Clin Oncol*. Jun 10
511   2018;36(17):1769-1770. doi:10.1200/jco.2018.78.9867

512   14. Cross NCP, Godfrey AL, Cargo C, Garg M, Mead AJ. The use of genetic tests to diagnose and manage
513   patients with myeloproliferative and myeloproliferative/myelodysplastic neoplasms, and related
514   disorders. *Br J Haematol*. Nov 2021;195(3):338-351. doi:10.1111/bjh.17766

515   15. Aslan JE. Platelet Proteomes, Pathways, and Phenotypes as Informants of Vascular Wellness and
516   Disease. *Arterioscler Thromb Vasc Biol*. Mar 2021;41(3):999-1011. doi:10.1161/atvbaha.120.314647

517   16. Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. *Blood*.
518   Jul 30 2015;126(5):582-8. doi:10.1182/blood-2014-08-531582

519   17. Denorme F, Campbell RA. Procoagulant platelets: novel players in thromboinflammation. *Am J Physiol*
520   *Cell Physiol*. Oct 1 2022;323(4):C951-c958. doi:10.1152/ajpcell.00252.2022

521   18. Arellano-Rodrigo E, Alvarez-Larrán A, Reverter JC, Villamor N, Colomer D, Cervantes F. Increased
522   platelet and leukocyte activation as contributing mechanisms for thrombosis in essential
523   thrombocythemia and correlation with the JAK2 mutational status. *Haematologica*. Feb 2006;91(2):169-
524   75.

525   19. Thrombosis: a major contributor to the global disease burden. Isth Steering Committee for World
526   Thrombosis Day. *J Thromb Haemost*. Oct 2014;12(10):1580-90. doi:10.1111/jth.12698

527   20. Panova-Noeva M, Marchetti M, Buoro S, et al. JAK2V617F mutation and hydroxyurea treatment as
528   determinants of immature platelet parameters in essential thrombocythemia and polycythemia vera
529   patients. *Blood*. Sep 1 2011;118(9):2599-601. doi:10.1182/blood-2011-02-339655

530   21. Shen Z, Du W, Perkins C, et al. Platelet transcriptome identifies progressive markers and potential
531   therapeutic targets in chronic myeloproliferative neoplasms. *Cell Rep Med*. Oct 19 2021;2(10):100425.
532   doi:10.1016/j.xcrm.2021.100425

533   22. Koschmieder S, Chatain N. Role of inflammation in the biology of myeloproliferative neoplasms. *Blood*
534   *Rev*. Jul 2020;42:100711. doi:10.1016/j.blre.2020.100711

535   23. Almanza A, Carlesso A, Chintha C, et al. Endoplasmic reticulum stress signalling - from basic
536   mechanisms to clinical applications. *FEBS J*. 2019 2019;286(2):241-278. doi:10.1111/febs.14608

537   24. Costa-Mattioli M, Walter P. The integrated stress response: From mechanism to disease. *Science*.
538   2020/04/24/ 2020;368(6489)doi:10.1126/science.aat5314

539   25. Čokić VP, Mossuz P, Han J, et al. Microarray and Proteomic Analyses of Myeloproliferative Neoplasms
540   with a Highlight on the mTOR Signaling Pathway. *PLoS One*. 2015;10(8):e0135463.
541   doi:10.1371/journal.pone.0135463

542 26. Tognon R, Almeida ESDC, Andraos-Rey R, et al. A proteomic study of myeloproliferative neoplasms
543   using reverse-phase protein arrays. *Leuk Lymphoma*. Dec 2020;61(13):3052-3065.
544   doi:10.1080/10428194.2020.1805110

545 27. Bartalucci N, Tozzi L, Bogani C, et al. Co-targeting the PI3K/mTOR and JAK2 signalling pathways
546   produces synergistic activity against myeloproliferative neoplasms. *J Cell Mol Med*. Nov
547   2013;17(11):1385-96. doi:10.1111/jcmm.12162

548 28. Ibarra J, Elbanna YA, Kurylowicz K, et al. Type I but Not Type II Calreticulin Mutations Activate the
549   IRE1α/XBP1 Pathway of the Unfolded Protein Response to Drive Myeloproliferative Neoplasms. *Blood*
550   *Cancer Discov*. Jul 6 2022;3(4):298-315. doi:10.1158/2643-3230.Bcd-21-0144

551 29. Kong G, You X, Wen Z, et al. Downregulating Notch counteracts Kras(G12D)-induced ERK activation
552   and oxidative phosphorylation in myeloproliferative neoplasm. *Leukemia*. Mar 2019;33(3):671-685.
553   doi:10.1038/s41375-018-0248-0

554 30. Saenz DT, Fiskus W, Mill CP, et al. Mechanistic basis and efficacy of targeting the β-catenin-TCF7L2-
555   JMJD6-c-Myc axis to overcome resistance to BET inhibitors. *Blood*. Apr 9 2020;135(15):1255-1269.
556   doi:10.1182/blood.2019002922

557 31. Haider S, Pal R. Integrated analysis of transcriptomic and proteomic data. *Curr Genomics*. Apr
558   2013;14(2):91-110. doi:10.2174/1389202911314020003

559 32. Buccitelli C, Selbach M. mRNAs, proteins and the emerging principles of gene expression control. *Nat*
560   *Rev Genet*. Oct 2020;21(10):630-644. doi:10.1038/s41576-020-0258-4

561 33. Karczewski KJ, Snyder MP. Integrative omics for health and disease. *Nat Rev Genet*. May
562   2018;19(5):299-310. doi:10.1038/nrg.2018.4

563 34. Gao A, Xu S, Li Q, et al. Interlukin-4 weakens resistance to stress injury and megakaryocytic
564   differentiation of hematopoietic stem cells by inhibiting Psmd13 expression. *Sci Rep*. Aug 31
565   2023;13(1):14253. doi:10.1038/s41598-023-41479-6

566 35. Eisa NH, Jilani Y, Kainth K, et al. The co-chaperone UNC45A is essential for the expression of mitotic
567   kinase NEK7 and tumorigenesis. *J Biol Chem*. Apr 5 2019;294(14):5246-5260.
568   doi:10.1074/jbc.RA118.006597

569 36. Hua YQ, Zhang K, Sheng J, et al. NUCB1 Suppresses Growth and Shows Additive Effects With
570   Gemcitabine in Pancreatic Ductal Adenocarcinoma via the Unfolded Protein Response. *Front Cell Dev*
571   *Biol*. 2021;9:641836. doi:10.3389/fcell.2021.641836

572 37. Maggiorani D, Manzella N, Edmondson DE, et al. Monoamine Oxidases, Oxidative Stress, and Altered
573   Mitochondrial Dynamics in Cardiac Ageing. *Oxid Med Cell Longev*. 2017;2017:3017947.
574   doi:10.1155/2017/3017947

23

575 38. Zhang J, Zhao ZJ, Fu X, et al. Proapoptotic Mitochondrial Carrier Homolog Protein PSAP Mediates
576   Death Receptor 6 Induced Apoptosis. *J Alzheimers Dis*. 2020;74(4):1097-1106. doi:10.3233/jad-191086
577 39. Zaouali MA, Panisello A, Lopez A, et al. GSK3β and VDAC Involvement in ER Stress and Apoptosis
578   Modulation during Orthotopic Liver Transplantation. *Int J Mol Sci*. Mar 8
579   2017;18(3)doi:10.3390/ijms18030591
580 40. Wang K, Lin B, Brems JJ, Gamelli RL. Hepatic apoptosis can modulate liver fibrosis through TIMP1
581   pathway. *Apoptosis*. May 2013;18(5):566-77. doi:10.1007/s10495-013-0827-5
582 41. Cheng Q, Wei T, Jia Y, et al. Dendrimer-Based Lipid Nanoparticles Deliver Therapeutic FAH mRNA to
583   Normalize Liver Function and Extend Survival in a Mouse Model of Hepatorenal Tyrosinemia Type I.
584   *Adv Mater*. Dec 2018;30(52):e1805308. doi:10.1002/adma.201805308
585 42. Xiong Y, Si Y, Feng Y, Zhuo S, Cui B, Zhang Z. Prognostic value of lipid metabolism-related genes in
586   head and neck squamous cell carcinoma. *Immun Inflamm Dis*. Mar 2021;9(1):196-209.
587   doi:10.1002/iid3.379
588 43. Zhu Y, Gu L, Lin X, et al. Dynamic Regulation of ME1 Phosphorylation and Acetylation Affects Lipid
589   Metabolism and Colorectal Tumorigenesis. *Mol Cell*. Jan 2 2020;77(1):138-149.e5.
590   doi:10.1016/j.molcel.2019.10.015
591 44. Moliterno AR, Ginzburg YZ, Hoffman R. Clinical insights into the origins of thrombosis in
592   myeloproliferative neoplasms. *Blood*. Mar 4 2021;137(9):1145-1153. doi:10.1182/blood.2020008043
593 45. Tefferi A. Primary myelofibrosis: 2023 update on diagnosis, risk-stratification, and management. *Am J
594   Hematol*. May 2023;98(5):801-821. doi:10.1002/ajh.26857
595 46. Harrison CN, Nangalia J, Boucher R, et al. Ruxolitinib Versus Best Available Therapy for Polycythemia
596   Vera Intolerant or Resistant to Hydroxycarbamide in a Randomized Trial. *J Clin Oncol*. May 1
597   2023:Jco2201935. doi:10.1200/jco.22.01935
598 47. Huang J, Heemskerk JWM, Swieringa F. Combining human platelet proteomes and transcriptomes:
599   possibilities and challenges. *Platelets*. Dec 2023;34(1):2224454. doi:10.1080/09537104.2023.2224454
600 48. Solari FA, Krahn D, Swieringa F, et al. Multi-omics approaches to study platelet mechanisms. *Curr Opin
601   Chem Biol*. Apr 2023;73:102253. doi:10.1016/j.cbpa.2022.102253
602 49. De Wispelaere K, Freson K. The Analysis of the Human Megakaryocyte and Platelet Coding
603   Transcriptome in Healthy and Diseased Subjects. *Int J Mol Sci*. Jul 11
604   2022;23(14)doi:10.3390/ijms23147647
605 50. He F, Laranjeira ABA, Kong T, et al. Multiomic Profiling Reveals Metabolic Alterations Mediating
606   Aberrant Platelet Activity and Inflammation in Myeloproliferative Neoplasms. *bioRxiv*.
607   2022:2022.12.08.519689. doi:10.1101/2022.12.08.519689

608   51. Sharda AV, Bogue T, Barr A, Mendez LM, Flaumenhaft R, Zwicker JI. Circulating Protein Disulfide
609   Isomerase Is Associated with Increased Risk of Thrombosis in JAK2-Mutated Myeloproliferative
610   Neoplasms. *Clin Cancer Res*. Oct 15 2021;27(20):5708-5717. doi:10.1158/1078-0432.Ccr-21-1140
611   52. Moore SF, Hunter RW, Harper MT, et al. Dysfunction of the PI3 kinase/Rap1/integrin α(IIb)β(3) pathway
612   underlies ex vivo platelet hypoactivity in essential thrombocythemia. *Blood*. Feb 14 2013;121(7):1209-
613   19. doi:10.1182/blood-2012-05-431288
614   53. Pecci A, Necchi V, Barozzi S, et al. Particulate cytoplasmic structures with high concentration of
615   ubiquitin-proteasome accumulate in myeloid neoplasms. *J Hematol Oncol*. Jun 18 2015;8:71.
616   doi:10.1186/s13045-015-0169-6
617   54. Pourcelot E, Trocme C, Mondet J, Bailly S, Toussaint B, Mossuz P. Cytokine profiles in polycythemia
618   vera and essential thrombocythemia patients: clinical implications. *Exp Hematol*. May 2014;42(5):360-8.
619   doi:10.1016/j.exphem.2014.01.006
620   55. Randi ML, Brunati AM, Scapin M, et al. Src tyrosine kinase preactivation is associated with platelet
621   hypersensitivity in essential thrombocythemia and polycythemia vera. *Blood*. Jan 21 2010;115(3):667-
622   76. doi:10.1182/blood-2008-12-196535
623   56. Fel A, Lewandowska AE, Petrides PE, Wiśniewski JR. Comparison of Proteome Composition of Serum
624   Enriched in Extracellular Vesicles Isolated from Polycythemia Vera Patients and Healthy Controls.
625   *Proteomes*. May 6 2019;7(2)doi:10.3390/proteomes7020020
626   57. Krishnan A, Thomas S. Toward platelet transcriptomics in cancer diagnosis, prognosis and therapy. *Br
627   J Cancer*. Feb 2022;126(3):316-322. doi:10.1038/s41416-021-01627-z
628   58. García Á. Platelet clinical proteomics: Facts, challenges, and future perspectives. *Proteomics Clin Appl*.
629   Aug 2016;10(8):767-73. doi:10.1002/prca.201500125
630   59. Geyer PE, Holdt LM, Teupser D, Mann M. Revisiting biomarker discovery by plasma proteomics. *Mol
631   Syst Biol*. Sep 26 2017;13(9):942. doi:10.15252/msb.20156297
632   60. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization
633   classification of myeloid neoplasms and acute leukemia. *Blood*. May 19 2016;127(20):2391-405.
634   doi:10.1182/blood-2016-03-643544
635   61. Cortelazzo S, Marchetti M, Orlando E, Falanga A, Barbui T, Buchanan MR. Aspirin increases the
636   bleeding side effects in essential thrombocythemia independent of the cyclooxygenase pathway: role of
637   the lipoxygenase pathway. *Am J Hematol*. Apr 1998;57(4):277-82. doi:10.1002/(sici)1096-
638   8652(199804)57:4<277::aid-ajh2>3.0.co;2-r
639   62. Parsons MEM, Szklanna PB, Guerrero JA, et al. Platelet Releasate Proteome Profiling Reveals a Core
640   Set of Proteins with Low Variance between Healthy Adults. *Proteomics*. Aug 2018;18(15):e1800219.
641   doi:10.1002/pmic.201800219

642   63. Szklanna PB, Parsons ME, Wynne K, et al. The Platelet Releasate is Altered in Human Pregnancy.
643   *Proteomics Clin Appl*. May 2019;13(3):e1800162. doi:10.1002/prca.201800162
644   64. Weiss L, Keaney J, Szklanna PB, et al. Nonvalvular atrial fibrillation patients anticoagulated with
645   rivaroxaban compared with warfarin exhibit reduced circulating extracellular vesicles with attenuated
646   pro-inflammatory protein signatures. *J Thromb Haemost*. Oct 2021;19(10):2583-2595.
647   doi:10.1111/jth.15434
648   65. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based
649   approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. Oct 25
650   2005;102(43):15545-50. doi:10.1073/pnas.0506580102
651   66. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures
652   Database (MSigDB) hallmark gene set collection. *Cell Syst*. Dec 23 2015;1(6):417-425.
653   doi:10.1016/j.cels.2015.12.004
654   67. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data
655   with DESeq2. *Genome Biol*. 2014;15(12):550. doi:10.1186/s13059-014-0550-8
656   68. Perez-Riverol Y, Bai J, Bandla C, et al. The PRIDE database resources in 2022: a hub for mass
657   spectrometry-based proteomics evidences. *Nucleic Acids Res*. Jan 7 2022;50(D1):D543-d552.
658   doi:10.1093/nar/gkab1038
659   69. Austin KM, Covic L, Kuliopulos A. Matrix metalloproteases and PAR1 activation. *Blood*. Jan 17
660   2013;121(3):431-9. doi:10.1182/blood-2012-09-355958
661   70. Trivedi V, Boire A, Tchernychev B, et al. Platelet matrix metalloprotease-1 mediates thrombogenesis by
662   activating PAR1 at a cryptic ligand site. *Cell*. Apr 17 2009;137(2):332-43. doi:10.1016/j.cell.2009.02.018
663   71. Mastenbroek TG, Feijge MA, Kremers RM, et al. Platelet-Associated Matrix Metalloproteinases
664   Regulate Thrombus Formation and Exert Local Collagenolytic Activity. *Arterioscler Thromb Vasc Biol*.
665   Dec 2015;35(12):2554-61. doi:10.1161/atvbaha.115.306153
666   72. Brinckerhoff CE, Rutter JL, Benbow U. Interstitial collagenases as markers of tumor progression. *Clin
667   Cancer Res*. Dec 2000;6(12):4823-30.
668   73. Nguyen CH, Senfter D, Basilio J, et al. NF-κB contributes to MMP1 expression in breast cancer
669   spheroids causing paracrine PAR1 activation and disintegrations in the lymph endothelial barrier in
670   vitro. *Oncotarget*. Nov 17 2015;6(36):39262-75. doi:10.18632/oncotarget.5741
671   74. Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A. PAR1 is a matrix metalloprotease-1
672   receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell*. Feb 11
673   2005;120(3):303-13. doi:10.1016/j.cell.2004.12.018
674   75. Arman M, Krauel K. Human platelet IgG Fc receptor FcγRIIA in immunity and thrombosis. *J Thromb
675   Haemost*. Jun 2015;13(6):893-908. doi:10.1111/jth.12905

76. Thienel M, Müller-Reif JB, Zhang Z, et al. Immobility-associated thromboprotection is conserved across mammalian species from bear to human. *Science*. Apr 14 2023;380(6641):178-187. doi:10.1126/science.abo5044

77. Kaiser WJ, Holbrook LM, Tucker KL, Stanley RG, Gibbins JM. A functional proteomic method for the enrichment of peripheral membrane proteins reveals the collagen binding protein Hsp47 is exposed on the surface of activated human platelets. *J Proteome Res*. Jun 2009;8(6):2903-14. doi:10.1021/pr900027j

78. Sasikumar P, AlOuda KS, Kaiser WJ, et al. The chaperone protein HSP47: a platelet collagen binding protein that contributes to thrombosis and hemostasis. *J Thromb Haemost*. May 2018;16(5):946-959. doi:10.1111/jth.13998

79. Giordano M, Croci DO, Rabinovich GA. Galectins in hematological malignancies. *Curr Opin Hematol*. Jul 2013;20(4):327-35. doi:10.1097/MOH.0b013e328362370f

80. Li R, Colombo M, Wang G, et al. A pro-inflammatory stem cell niche drives myelofibrosis through a targetable galectin 1 axis. *bioRxiv*. 2023:2023.08.05.550630. doi:10.1101/2023.08.05.550630

81. Diklić M, Mitrović-Ajtić O, Subotički T, et al. IL6 inhibition of inflammatory S100A8/9 proteins is NF-κB mediated in essential thrombocythemia. *Cell Biochem Funct*. Jun 2020;38(4):362-372. doi:10.1002/cbf.3482

82. Leimkühler NB, Costa IG, Schneider RK. From cell to cell: Identification of actionable targets in bone marrow fibrosis using single-cell technologies. *Exp Hematol*. Dec 2021;104:48-54. doi:10.1016/j.exphem.2021.09.006

83. Wang S, Song R, Wang Z, Jing Z, Wang S, Ma J. S100A8/A9 in Inflammation. *Front Immunol*. 2018;9:1298. doi:10.3389/fimmu.2018.01298

84. Wang YH, Chen YJ, Lai YH, et al. Mutation-Driven S100A8 Overexpression Confers Aberrant Phenotypes in Type 1 CALR-Mutated MPN. *Int J Mol Sci*. May 14 2023;24(10)doi:10.3390/ijms24108747

85. Kovačić M, Mitrović-Ajtić O, Beleslin-Čokić B, et al. TLR4 and RAGE conversely mediate pro-inflammatory S100A8/9-mediated inhibition of proliferation-linked signaling in myeloproliferative neoplasms. *Cell Oncol (Dordr)*. Oct 2018;41(5):541-553. doi:10.1007/s13402-018-0392-6

86. Lay AJ, Dupuy A, Hagimola L, et al. Endoplasmic reticulum protein 5 attenuates platelet endoplasmic reticulum stress and secretion in a mouse model. *Blood Adv*. May 9 2023;7(9):1650-1665. doi:10.1182/bloodadvances.2022008457

87. Yang M, Chiu J, Scartelli C, et al. Sulfenylation links oxidative stress to protein disulfide isomerase oxidase activity and thrombus formation. *J Thromb Haemost*. Aug 2023;21(8):2137-2150. doi:10.1016/j.jtha.2023.03.034

88. Pan J, Lordier L, Meyran D, et al. The formin DIAPH1 (mDia1) regulates megakaryocyte proplatelet formation by remodeling the actin and microtubule cytoskeletons. *Blood*. Dec 18 2014;124(26):3967-77. doi:10.1182/blood-2013-12-544924

89. Shirakawa R, Yoshioka A, Horiuchi H, Nishioka H, Tabuchi A, Kita T. Small GTPase Rab4 regulates Ca2+-induced alpha-granule secretion in platelets. *J Biol Chem*. Oct 27 2000;275(43):33844-9. doi:10.1074/jbc.M002834200

90. Yu S, Chen J, Quan M, Li L, Li Y, Gao Y. CD63 negatively regulates hepatocellular carcinoma development through suppression of inflammatory cytokine-induced STAT3 activation. *J Cell Mol Med*. Jan 2021;25(2):1024-1034. doi:10.1111/jcmm.16167

91. Alam S, Liu Q, Liu S, et al. Up-regulated cathepsin C induces macrophage M1 polarization through FAK-triggered p38 MAPK/NF-κB pathway. *Exp Cell Res*. Sep 15 2019;382(2):111472. doi:10.1016/j.yexcr.2019.06.017

92. Joshi S, Banerjee M, Zhang J, et al. Alterations in platelet secretion differentially affect thrombosis and hemostasis. *Blood Adv*. Sep 11 2018;2(17):2187-2198. doi:10.1182/bloodadvances.2018019166

93. Li K, Tan G, Zhang X, et al. EIF4G1 Is a Potential Prognostic Biomarker of Breast Cancer. *Biomolecules*. Nov 26 2022;12(12)doi:10.3390/biom12121756

94. Albakova Z, Armeev GA, Kanevskiy LM, Kovalenko EI, Sapozhnikov AM. HSP70 Multi-Functionality in Cancer. *Cells*. Mar 2 2020;9(3)doi:10.3390/cells9030587

95. Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. *Nat Rev Cancer*. Aug 2010;10(8):537-49. doi:10.1038/nrc2887

96. Zhang J, Li H, Liu Y, et al. Targeting HSP90 as a Novel Therapy for Cancer: Mechanistic Insights and Translational Relevance. *Cells*. Sep 6 2022;11(18)doi:10.3390/cells11182778

97. Bennett JA, Mastrangelo MA, Ture SK, et al. The choline transporter Slc44a2 controls platelet activation and thrombosis by regulating mitochondrial function. *Nat Commun*. Jul 13 2020;11(1):3479. doi:10.1038/s41467-020-17254-w

98. Germain M, Chasman DI, de Haan H, et al. Meta-analysis of 65,734 individuals identifies TSPAN15 and SLC44A2 as two susceptibility loci for venous thromboembolism. *Am J Hum Genet*. Apr 2 2015;96(4):532-42. doi:10.1016/j.ajhg.2015.01.019

99. Ajanel A, Campbell RA, Denorme F. Platelet mitochondria: the mighty few. *Curr Opin Hematol*. Sep 1 2023;30(5):167-174. doi:10.1097/moh.0000000000000772

100. Aguilar A, Weber J, Boscher J, et al. Combined deficiency of RAB32 and RAB38 in the mouse mimics Hermansky-Pudlak syndrome and critically impairs thrombosis. *Blood Adv*. Aug 13 2019;3(15):2368-2380. doi:10.1182/bloodadvances.2019031286

743   101.   Osorio FG, Soria-Valles C, Santiago-Fernández O, et al. Loss of the proteostasis factor AIRAPL
744   causes myeloid transformation by deregulating IGF-1 signaling. *Nat Med*. Jan 2016;22(1):91-6.
745   doi:10.1038/nm.4013
746   102.   LaFave LM, Levine RL. Targeting a regulator of protein homeostasis in myeloproliferative
747   neoplasms. *Nat Med*. Jan 2016;22(1):20-1. doi:10.1038/nm.4028
748   103.   Jutzi JS, Marneth AE, Jiménez-Santos MJ, et al. CALR-mutated cells are vulnerable to combined
749   inhibition of the proteasome and the endoplasmic reticulum stress response. *Leukemia*. Feb
750   2023;37(2):359-369. doi:10.1038/s41375-022-01781-0

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Table 1A and B Description

Table 1A: MPN patient characteristics recruited across two sites (Papa Giovanni XXIII Hospital, Bergamo, Italy and Mater Misericordiae University Hospital, Dublin, Ireland) for proteomic analysis.

Table 1B: MPN patient characteristics recruited at single center (Stanford, USA[21]) included here for comparative transcriptomic analysis. Key demographic and other patient variables (listed below) have no statistically significant difference (unpaired t-test) between the (transcriptome) and Mater/Bergamo proteome cohorts. These variables include: age, sex, platelet count, and distribution of MPN driver mutation (*JAK2, CALR, MPL*) and therapy (aspirin, hydroxyurea).

Table 2 Description

Select/representative candidate proteins that may variably influence the proinflammatory, pro-thrombotic, and profibrotic processes in MPNs.

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801   **Figure Legends**

802

803   **Figure 1: Patient variables representing MPN clinical cohort for proteomic work package**

804   **A,** Similarity in distribution of MPN subtypes and controls, with slightly higher proportion ET (PV n= 41, ET n=

805   59, control n= 40) **B**, Comparable and balanced distribution of sex across MPN subtypes and controls. Larger

806   percentage of female healthy controls. **C,** All patients with PV harbored the *JAK2* V617F mutation, and in

807   keeping with the general ET population *JAK2* V617F was the most common driver mutation followed by

808   *CALR* and *MPL*, with 12 triple negative ET patients included in this study. **D,** MPN patient therapies reflecting

809   current clinical practice. The majority of PV and ET patients were prescribed aspirin (ASA), with hydroxyurea

810   (HU) as a commonly utilized cytoreductive therapy. To control for any inter-patient variability, all treatment, in

811   addition to patient, sex and experimental batch are adjusted as confounding factors in downstream differential

812   expression analyses **E,** Comparable distribution of age across MPN subtypes and controls. Violin plots of

813   patient age from each MPN subtype reflect clinical expectation, with slightly higher median age noted for ET

814   and PV patients compared to controls. **F,** Platelet counts, as box plots, measured at the same date and time

815   as experimental platelet sampling. As expected, Mann-Whitney U tests marked by asterisks indicate a

816   statistically significant difference between control and MPN groups (*** p= <0.001, ns = not significant).

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818   **Figure 2: MPN platelet proteome distinguishes disease phenotype and reveals subtype specific**

819   **signatures**

820   **A,** Unsupervised principal component analysis (PCA) of normalized platelet protein expression adjusted for

821   age, sex, treatment (antiplatelet and cytoreduction) and experimental batch. PC1 and PC2 colored by MPN

822   subtype; and each contrasted with controls (n= 40, yellow): ET (n= 59, ET (n= 59, light green), PV (n= 41,

823   dark green). The first two principal components account for 26% of total variance in the data. **B,** Volcano plots

824   (two panels of ET, PV) of differential protein expression showing log2 fold change versus statistical

825   significance (negative log10 of p-values) of each gene. Significant up-regulated and down-regulated genes

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826 are those with *p*-values (FDR) smaller or equal to 0.05 and absolute value of fold changes larger or equal to

827 1.5**.**

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829 **Figure 3: The platelet proteome in MPN identifies biological pathways recognised as crucial drivers**

830 **of disease**

831 **A,** Pathway-enrichment analysis of proteins with MPN subtype-specific expression (color indicated; yellow

832 ET, and light green PV). Each point represents a pathway; the *x*-axis gives the normalized enrichment score,

833 which reflects the degree to which each pathway is over-represented at the top of the ranked list of

834 differentially expressed proteins, normalized to account for differences in gene set size and in correlations

835 between gene sets and the expression data set. The y-axis lists the detail-level node of the most enriched

836 pathways; solid lines mark GSEA-recommended[65] Bonferroni-corrected statistical significance criterion of

837 FDR < 0.25 for exploratory analyses.

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839 **Figure 4: Correlation of differential expression between a shared set of significant genes and proteins**

840 Spearman's correlation showed moderate (Rho = 0.44, *p* = 3.484e-11) correlation in differential expression

841 (log2FC) of genes from the MPN platelet transcriptomic cohort that were also significantly differentially

842 expressed (FDR < 0.05) at the MPN platelet proteomic cohort.

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853 **Figure 5: Highly expressed shared platelet proteo-transcriptome features in MPNs (A & B)**

854 Taking the overlap of the significantly differentially expressed proteins and genes (FDR < 0.05) that were

855 common across MPN subtypes (i.e., present in both ET and PV samples), we identified highly expressed

856 features (n= 17) shared between the two independent cohorts. Hierarchically clustered heatmaps of the

857 overlapping features between significantly expressed protein and genes (FDR <0.05) from controls versus

858 MPN (present in both PV and ET). **A,** Transcriptomic expression from the Stanford single center cohort (n=

859 78). **B,** Proteomic expression from the international, multi-center collaborative (Ireland & Italy: n= 140).

860 Colored annotation is provided to indicate MPN subtype, mutation status and sex. Rows indicate gradation in

861 expression on a yellow (low) to orange (high) scale. Columns indicate sample type from controls, ET, and PV.

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878 **Supplementary Figures**

879 **Figure S1: Strong correlation between platelet proteome technical replicates.**

880 Pearson correlation coefficients (*r*) from representative samples **(A-D)** demonstrate intra-donor reproducibility

881 with strong correlation of log transformed LFQ intensities from technical replicates of the platelet proteome.

882

883 **Figure S2: Strong correlation between platelet proteome biologic replicates**

884 Correlation matrix of Pearson correlation coefficients (*r*) from biologic replicates show uniformly strong inter-

885 donor reproducibility of the LFQ-proteomic analysis between biologic replicates from **(A)** controls (average *r*

886 = 0.90 ± 0.04, min *r* = 0.75, max *r*= 0.97) and **(B)** MPN (average *r* = 0.88 ± 0.06, min *r* = 0.57, max *r* = 0.98)

887 patient samples.

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Supplementary Tables

Table S1: 1952 proteins were quantified (LFQ intensity, see Methods) across PV, ET and control platelet lysate samples.

Table S2: 1315 proteins taken forward for downstream analysis. Proteins filtered to remove contaminants, proteins identified by site only, or in reverse. Proteins included were quantified across all groups (PV, ET and control) and were present in at least 50% of samples in at least one group (see Methods).

Table S3: Full list of 227 differentially expressed platelet proteins (Benjamini Hochberg false discovery rate <0.05) identified between ET and control samples.

Table S4: Full list of 166 differentially expressed platelet proteins (Benjamini Hochberg false discovery rate <0.05) were identified between PV and control samples.

Table S5: 1771 proteins quantified across technical replicate samples (n= 6).

Table S6: Full data for all molecular pathways identified in platelet proteome of ET patient cohorts.

Table S7: Full data for all molecular pathways identified in platelet proteome of PV patient cohorts.

Table S8: Correlation matrix with Pearson correlation coefficient *(r)* of log2 transformed LFQ intensity from biologic replicates of control (n= 40) samples.

Table S9: Correlation matrix with Pearson correlation coefficient *(r)* of log2 transformed LFQ intensity from biologic replicates of MPN (PV n= 41; ET n= 59) samples.
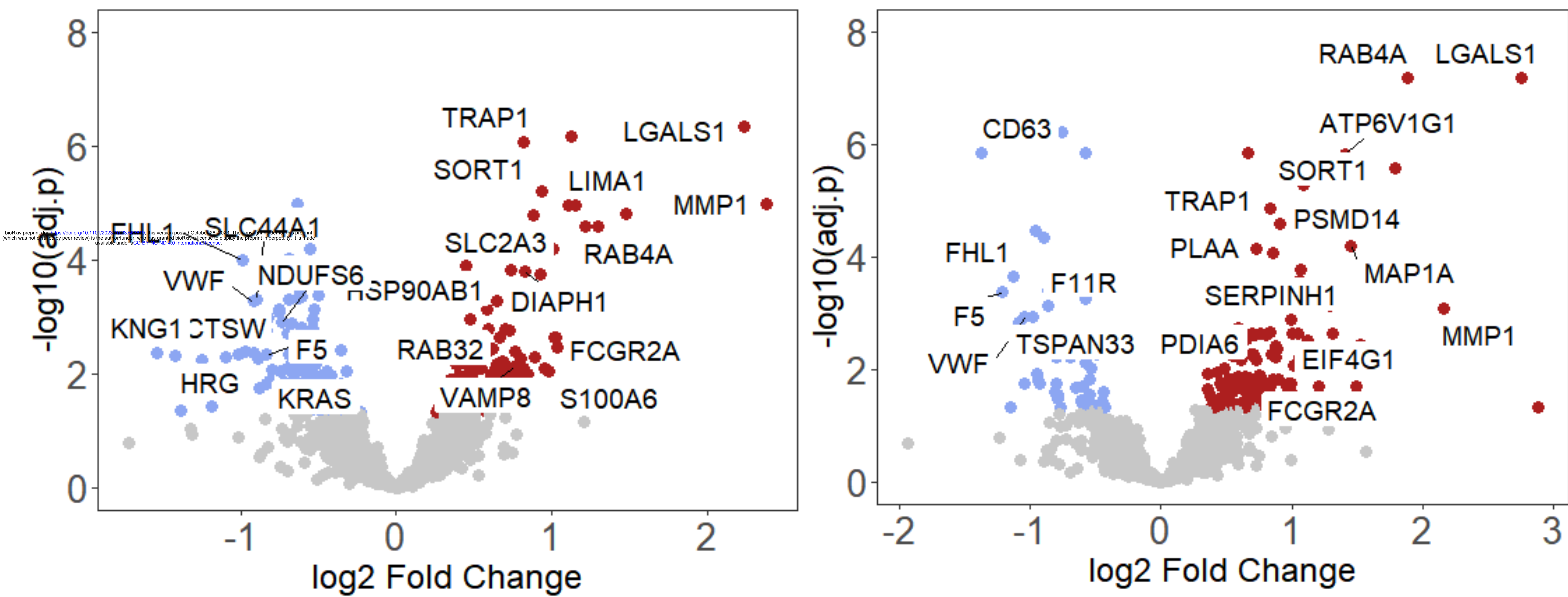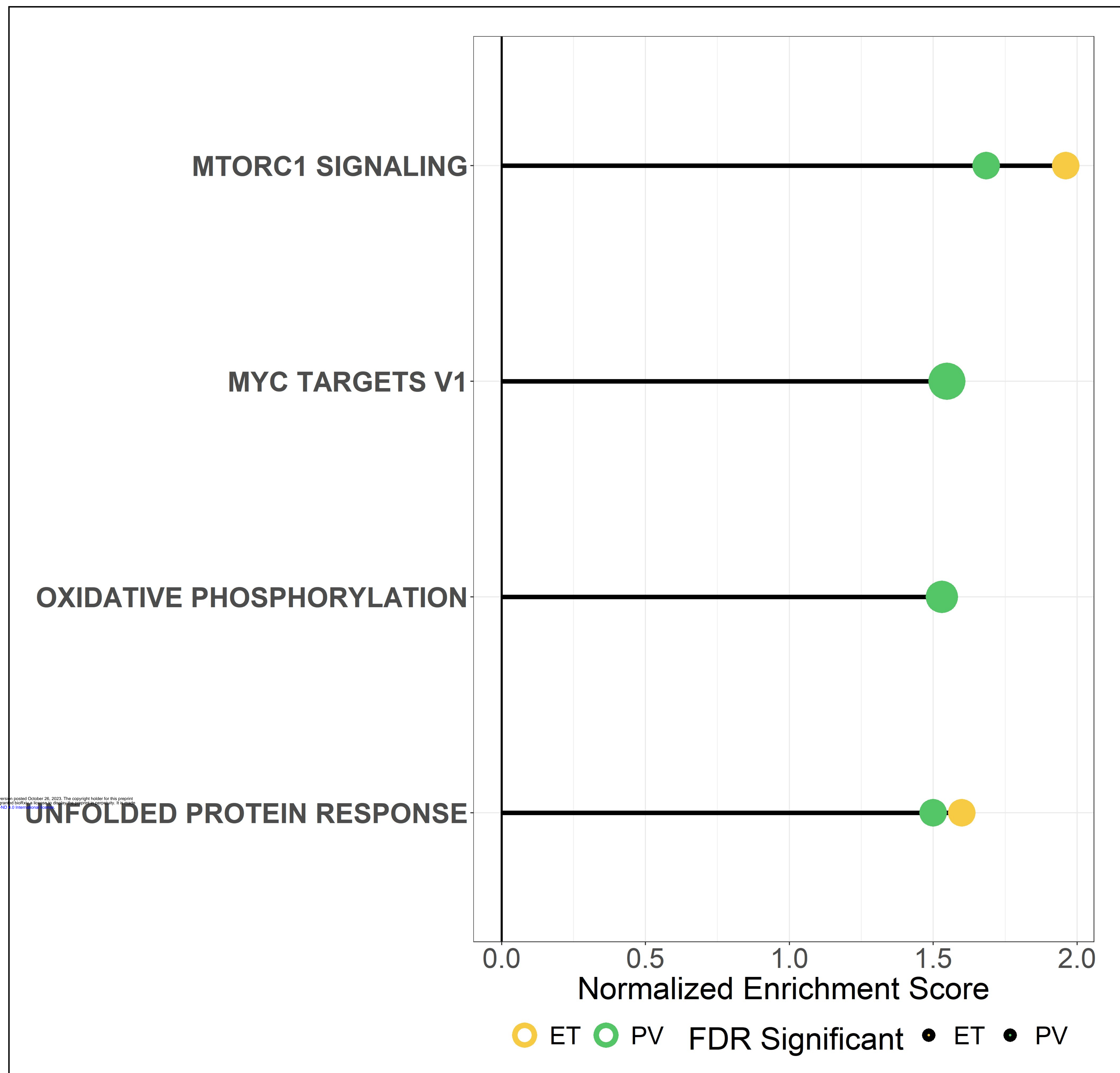
FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

Spearman's rho = 0.44
p-value = 3.484e-11

LGALS1
ME1
ATP6V1B2
LIMA1
MAOB
FCGR2A
CUL2
ATP2A2
TUBA8
ANXA3
SH3BGRL2
HDHD2
F5
SNTB1
C11orf54
VWF
ALAD

Platelet Proteome (log2 fold change)

Platelet Transcriptome (log2 fold change)

FIGURE 5

Figure S1

Figure S2