Supplementary Methods

Sample preparation for proteomics measurements:

For mass spectrometry (MS) analysis, 25,000 cells per sample were sorted into protein low-binding micro-centrifuge tubes (Eppendorf) containing 300 µl PBS buffer. The remaining material was sorted into separate micro-centrifuge tubes for spectral library generation. Depending on patient sample and cell type, this resulted in 19 additional samples (9x HSC/MPP, 6x GMP, 3x CMP, 1x MEP) with 47,860 to 91,749 cells (see supplementary table S3). Sorted cells were pelleted by centrifugation at 400 g for 12 min at 4°C. The supernatant was carefully removed, leaving ~30 µl on top of the pellet to avoid losing the non-adherent cell pellet. The pelleted cells were then snap frozen in liquid nitrogen. The remaining FACS buffer was lyophilized.

The lyophilized cell pellets were resuspended in 10 µl of 8 M urea in 100 mM ammonium hydrogen carbonate. Cells were lysed by sonication with a VialTweeter (Hielscher) at an amplitude of 60%, a cycle of 60% and a duration of 20 s for 3 times with intermediate cooling on ice. Protein content and amount of protease to be added was determined by submitting 100,000 CD34+ hematopoietic stem/progenitor cells to a BCA protein assay following the manufacturer's instructions (Pierce/ThermoFisher). Samples were diluted to 4 M urea with 100 mM ammonium hydrogen carbonate. They were treated with 1.25 U Benzonase Nuclease (Sigma-Aldrich) per 25,000 cells for 30 min at 37 °C. Reduction of disulfide bonds was performed by adding tris-(2-carboxyethyl)-phosphine (Sigma-Aldrich) to 5 mM and incubating at 37 °C for 30 min with shaking in a Thermomixer (Eppendorf). Alkylation of free thiol groups was performed by adding iodoacetamide (Sigma-Aldrich) to 10 mM in the dark for 30 min at room temperature. Samples were then diluted to 1 M urea and digested. Sequencing grade trypsin (Promega) was added at an enzyme-to-substrate ratio of 1:50 (extrapolated from the BCA assay results) for an overnight digestion at 37 °C. We adjusted samples to 2 % formic acid and desalted them with Empore Disks C18 (3M) self-packed into StageTips format.[1] After drying in a vacuum centrifuge, samples were resuspended in 11 µl of 2 % acetonitrile and 0.1% formic acid with the addition of iRT peptides (Biognosys) for the following MS analysis.[2] Peptide concentrations were determined using the quantitative fluorimetric peptide assay (Pierce/Thermo Scientific) following the manufacturer's guidelines.

Mass spectrometry analysis:

Nanoflow LC-MS/MS measurements were carried out on an EASY-nLC 1200 system (Thermo Scientific) connected to an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific) with a Nanospray Flex ion source.

Individual patient/control sample separation:

Peptide separation was performed on an Acclaim PepMap 100 C18 column (Thermo Scientific) with an inner diameter of 75 µm, a length of 25 cm, and a particle size of 2 µm. We operated the column at room temperature and at a flow rate of 300 nl/min. LC solvent A was composed of 98 % water, 2 % acetonitrile and 0.1 % formic acid. LC solvent B consisted of 80 % acetonitrile, 20 % water and 0.1 % formic acid. Peptides for individual measurements of patient/control samples were separated by a linear gradient from 5 to 37 % B over 120 min.

Library sample separation:

Peptides from patients/controls for spectral library generation were separated by a linear gradient from 5 to 37 % B over 240 min. In addition to the patient/control sample library, a HEK293 spectral library was generated. 80 µg peptides from HEK293 cells were treated by high pH reversed-phase peptide fractionation using a dedicated spin column kit (Pierce/Thermo Scientific) and following manufacturer's guidelines. Briefly, we collected 8 fractions, eluting with 300 µl with increasing acetonitrile content from 5 % to 50 % in 0.1 % trimethylamine. The fractions were dried in a vacuum centrifugation and redissolved in 25 µl of 2 % acetonitrile, 0.1 % formic acid with iRT peptides. 2 µl were injected per measurement. In addition, unfractionated whole lysates of HEK293 were measured by LC-MS (see supplementary table S3). Peptides from HEK293 cells for library generation were separated over 240 min for unfractionated samples and 180 min for high pH reversed phase fractions, respectively.

MS instrument settings:

The MS instrument was operated either in the DDA (library) or DIA (individual patient/control sample measurements) mode. In both cases, fragmentation was achieved by higher energy collision dissociation

(HCD) at a normalized collision energy setting of 27 %. The Orbitrap analyzer was set at a resolution of 120,000 and 30,000 for MS1 and MS2, with maximum injection times of 100 ms and 50 ms, respectively. For DIA, the mass range monitored in MS1 was 350-1500 m/z and 200-1800 m/z in MS2. For DDA, the auto m/z normal scan range mode was used. In MS1, we set the AGC target to 2e5. In MS2, we set the AGC target to 5e5 for DIA and 8e4 for DDA.
We used the top speed setting for DDA measurements and library generation: one MS1 survey scan was followed by the acquisition of MS/MS spectra for a cycle time of up to a maximum of 3 s. In DIA mode (individual patient/control sample measurements), one MS1 scan was followed by 40 MS2 windows of equal width (15 m/z) with an overlap of 1 m/z, covering precursors in the range of 399.5-1000.5 m/z. This led to a cycle time of 3.4 s. We acquired data with Xcalibur 4.0.27.10 and Tune Plus version 2.1.

Data analysis:

Library:

DDA data were searched by Mascot[3] (Matrix Science, version 2.5.1) and Comet[4] version 2016.01 rev. 2 against the Swissprot reviewed subset of the human UniProt database. The latter contained 20,199 protein entries and one additional protein entry for the concatenated iRT peptide sequences, as well as appended decoy sequences generated by sequence reversal (keeping C-terminal K and R residues). The following settings were applied: enzyme = trypsin, missed cleavages = ≤2, peptide tolerance = ±10 ppm, MS/MS tolerance = 0.02 Da, fixed modification = carbamidomethylation on cysteine, variable modification = oxidation on methionine.
Spectral libraries from DDA runs were generated as previously delinated.[5] Briefly, we independently adjusted the peptide-level false discovery rates (FDR) for the search outcomes to 1% for Mascot and Comet results using PeptideProphet (TPP v4.7 rev 0).[6,7] Mascot and Comet results were merged by iProphet[8] and filtered to 1% protein FDR by Mayu[9] (v 1.08). We used SpectraST[10,11] (v 5.0) to compile a consensus library for the query of the DIA measurements with the following filter criteria: include peptides at 1% peptide FDR for proteins at 1% protein FDR, 6 fragments per peptide, and fragment m/z range of 350 - 1800 m/z. The HEK293 library comprised 112,227 peptide precursor entries from 9,127 protein groups. The combined library for HEK293 and hematopoietic stem and progenitor cells included 146,610 peptide precursors from 10,057 protein groups.

Individual patient/control sample measurements:

DIA data were assessed by Spectronaut 11[12] (Biognosys) querying the library mentioned above with the following settings: data extraction with a tolerance of 10 ppm and 25 ppm for MS1 and MS2 level, with a dynamic retention time extraction window and automatic non-linear iRT retention time calibration, identification at a precursor Q-value cutoff of 0.01 and protein Q-value of 0.01, quantification based on MS2 level area, without cross-run normalization. We counted protein groups as defined by Spectronaut and exported the Spectronaut report (information on precursor, not fragment level) for further processing in R. All R scripts are deposited on github and can be accessed under
https://github.com/ge11232002/p2378.

Table S1

PROTEOMIC/TRANSCRIPTOMIC ANALYSES

Group	Subgroup	Patient ID	Age	Sex	Diagnosis	*JAK2*-V617 allele burden	Therapy
PV	PVchron.UT	PV1	57	m	PV		ASA, BP
PV	PVchron.UT	PV5	59	m	PV	43%	ASA
PV	PVchron.UT	PV6	53	m	PV	93%	ASA
PV	PVchron.UT	PV8	68	f	PV		ASA, BP, statin
PV	PVchron.UT	PV9	55	m	PV	92%	ASA, BP
PV	PVchron.UT	PV12	44	m	PV		ASA, BP
PV	PVchron.UT	PV13	55	m	PV	78%	ASA, BP
PV	PVchron.UT	PV14	44	f	PV	97%	ASA, anti-dep
PV	PVchron.UT	PV16	50	m	PV	43%	ASA
PV	PVchron.HU	PV2	65	m	PV		HU, ASA, BP, statin
PV	PVchron.HU	PV3	77	m	PV	95%	HU, ASA, BP
PV	PVchron.HU	PV4	57	m	PV	4%	HU, ASA, BP
PV	PVchron.HU	PV5	60	m	PV	43%	HU, ASA
PV	PVchron.HU	PV7	79	m	PV		HU, ASA, BP, anti-epi
PV	PVprog.HU	PV10	65	m	PV transforming into AML		HU, ASA
PV	PVprog.HU	PV11	61	m	Post-PV MF	99%	HU, ASA
PV	PVprog.HU	PV15	82	m	Post-PV MF		HU, phenprocoumon, anti-uric
PV	PVprog.UT	PV17	64	m	Post-PV MF		BP, anti-uric, pred
PV	PVprog.UT	PV18	72	f	PV transforming into MF	98%	ASA, BP, anti-uric
Control	Control	CON1	45-74	m,f	4 Healthy Donors		n.a.
Control	Control	CON2	45-74	m,f	4 Healthy Donors		n.a.
Control	Control	CON3	45-74	m,f	4 Healthy Donors		n.a.
Control	Control	CON4	71	m	Hemochromatosis		BP
Control	Control	CON5	42	m	Hemochromatosis		none
Control	Control	CON5	66	m	Hemochromatosis		ASA, BP, anti-dep
Control	Control	CON5	61	f	Hemochromatosis		inhal
Control	Control	CON5	40	m	Hemochromatosis		none
Control	Control	CON6	54	m	Hemochromatosis		ASA, anti-diab
Control	Control	CON7	45	m	Hemochromatosis		thyrox
Control	Control	CON7	60	f	Hemochromatosis		BP, HRT
Control	Control	CON8	63	m	Hemochromatosis		statin

VALIDATION EXPERIMENTS

Group	Subgroup	Patient ID	Age	Sex	Diagnosis	*JAK2*-V617 allele burden	Therapy
PV	PVchron.UT	PV6	47	m	PV	93%	ASA
PV	PVchron.UT	PV9	53	m	PV	92%	ASA, BP
PV	PVchron.UT	PV13	55	m	PV	78%	ASA
PV	PVchron.UT	PV14	43	f	PV	97%	ASA, Infliximab

PV	PVchron.UT	PV19	49	f	PV		Clopidogrel
PV	PVchron.UT	PV22	44	m	PV		ASA, BP
PV	PVchron.UT	PV23	55	m	PV		ASA
PV	PVprog.RU	PV11	62	m	Post-PV MF	99%	Ruxolitinib, ASA
PV	PVprog.UT	PV18	71	f	PV transforming into MF		ASA, BP
PV	PVprog.UT	PV20	77	m	Post-PV AML		ASA, BP, anti-uric
Control	Control	CON9	54	m	Healthy Donor		n.a.
Control	Control	CON10	55	m	Healthy Donor		n.a.
Control	Control	CON11	56	m	Healthy Donor		n.a.
Control	Control	CON12	74	m	Healthy Donor		n.a.
Control	Control	CON13	45	f	Healthy Donor		n.a.
Control	Control	CON14	53	f	Healthy Donor		n.a.
Control	Control	CON15	59	m	Healthy Donor		n.a.
Control	Control	CON16	46	f	Healthy Donor		n.a.
Control	Control	CON17	57	m	Healthy Donor		n.a.
Control	Control	CON18	53	m	Healthy Donor		n.a.
Control	Control	CON19	62	m	Healthy Donor		n.a.
Control	Control	CON20	65	f	Healthy Donor		n.a.

Table S1. Characteristics of the PV patients and controls studied.

For PV1/8/12, CON1, CON2 and CON3, samples from different individuals had to be pooled to guarantee adequate HSC/MPP numbers for downstream MS and RNA-seq measurements. Abbreviations: PV – polycythemia vera; MF – myelofibrosis; AML – acute myeloid leukemia; chron – chronic; prog – progressed; UT – untreated; HU – under treatment with hydroxyurea; RU – under treatment with ruxolitinib; ASA – acetylsalicylic acid; HU – hydroxyurea; BP – blood pressure medication; anti-dep – anti-depressive medication; anti-epi – anti-epileptic medication; anti-uric – uric acid lowering medication; pred – prednison; inhal – inhalative medication; anti-diab – anti-diabetic medication; thyrox – thyroxine medication; HRT – hormone replacement therapy; m – male; f – female.

Antibody	Clone	Provider	Order number
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD2	S5.5	ThermoFisherScientific-Invitrogen	CD0206
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD3	7D6	ThermoFisherScientific-Invitrogen	MHCD03065
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD4	S3.5	ThermoFisherScientific-Invitrogen	MHCD0406
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD7	CD7-6B7	ThermoFisherScientific-Invitrogen	MHCD0706
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD8	3B5	ThermoFisherScientific-Invitrogen	MHCD0806
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD14	TuK4	ThermoFisherScientific-Invitrogen	MHCD1406
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD19	SJ25-C1	ThermoFisherScientific-Invitrogen	MHCD1906
Tricolor/phycoerythrin (PE)-Cy5-conjugated anti-hCD56	MEM-188	ThermoFisherScientific-Invitrogen	MHCD5606
Phycoerythrin (PE)-Cy5 anti-hCD10	HI10a	BioLegend	312206
Phycoerythrin (PE)-Cy5 anti-hCD11b	ICRF44	BioLegend	301308
Phycoerythrin (PE)-Cy5 anti-hCD20	2H7	BioLegend	302308
Phycoerythrin (PE)-Cy5 anti-hCD235a	GA-R2	BD Biosciences	559944
PE-Cy7-conjugated anti-hCD34	8G12	BD Biosciences	348811
FITC-conjugated anti-hCD38	HIT2	BD Biosciences	555459
APC-conjugated anti-hCD123	6H6	ThermoFisherScientific-Invitrogen	17-1239-42
APC780-conjugated anti-hCD45RA	HI100	ThermoFisher Scientific-Invitrogen	47-0458-41
BV711-conjugated anti-hCD45RA	HI100	Biolegend	304138
PE-conjugated anti-hCLU	01	Sinobiological	11297-MM01_P
PE-conjugated anti-hLGALS9	9M1-3	BioLegend	348906
PE-conjugated monoclonal mouse IgG1 kappa	P3.6.2.8.1	ThermoFisherScientific-Invitrogen	12-4714-41
PE-conjugated monoclonal mouse IgG1 kappa	MOPC-21	BioLegend	400112

Table S2. Antibodies used in the study.

	Sample name	**Cell subpopulation**	**Cell number**
Patient samples			
samon_D1807_216	FMA 43	MEP	82728
samon_D1807_218	FMA 42	CMP	85686
samon_D1807_219	FMA 42	CMP	85686
samon_D1807_220	FMA 33	CMP	47860
samon_D1807_222	FMA 34	HSC/MPP	49395
samon_D1807_223	FMA 28	HSC/MPP	75711
samon_D1807_225	FMA 41	GMP	91749
samon_D1807_226	FMA 50	GMP	67210
samon_D1807_227	FMA 50	GMP	67210
samon_D1807_228	FMA 50	GMP	67210
samon_D1807_230	FMA 60	GMP	56250
samon_D1807_232	FMA 60	GMP	56250
samon_D1807_234	FMA 59	HSC/MPP	69767
samon_D1807_235	FMA 59	HSC/MPP	69767
samon_D1807_236	FMA 59	HSC/MPP	69767
samon_D1807_238	FMA 44	HSC/MPP	88297
samon_D1807_239	FMA 44	HSC/MPP	88297
samon_D1807_240	FMA 44	HSC/MPP	88297
samon_D1807_241	FMA 44	HSC/MPP	88297
Healthy control samples			
samon_D1704_035	236_CMP	CMP	45420
samon_D1704_036	234_CMP	CMP	52361
samon_D1704_038	236_MEP	MEP	43398
samon_D1704_039	235_MEP	MEP	57978
samon_D1704_041	236_HSC	HSC/MPP	64328
samon_D1704_042	236_HSC	HSC/MPP	64328
samon_D1704_044	235_GMP	GMP	48182
samon_D1704_045	235_GMP	GMP	48182
samon_D1704_047	185_MEP	MEP	71370
samon_D1704_048	185_MEP	MEP	71370
samon_D1704_050	234_HSC	HSC/MPP	71141
samon_D1704_051	234_HSC	HSC/MPP	71141
samon_D1704_053	185_GMP	GMP	70658
samon_D1704_054	185_GMP	GMP	70658
samon_D1704_056	185_HSC	HSC/MPP	89140
samon_D1704_057	185_HSC	HSC/MPP	89140
samon_D1704_059	235_CMP	CMP	91457
samon_D1704_060	235_CMP	CMP	91457
samon_D1704_062	185_CMP	CMP	60015
samon_D1704_063	185_CMP	CMP	60015
samon_D1704_064	185_CMP	CMP	60015
samon_D1704_065	185_CMP	CMP	60015
samon_D1704_067	235_HSC	HSC/MPP	67103
samon_D1704_068	235_HSC	HSC/MPP	67103

samon_D1704_069	235_HSC	HSC/MPP	67103
samon_D1704_070	235_HSC	HSC/MPP	67103
HEK cell samples			
samon_D1705_122	HEK highpH fr1		
samon_D1705_123	HEK highpH fr1		
samon_D1705_124	HEK highpH fr2		
samon_D1705_125	HEK highpH fr2		
samon_D1705_127	HEK highpH fr3		
samon_D1705_128	HEK highpH fr3		
samon_D1705_129	HEK highpH fr4		
samon_D1705_130	HEK highpH fr4		
samon_D1705_132	HEK highpH fr5		
samon_D1705_133	HEK highpH fr5		
samon_D1705_134	HEK highpH fr6		
samon_D1705_135	HEK highpH fr6		
samon_D1705_137	HEK highpH fr7		
samon_D1705_138	HEK highpH fr7		
samon_D1705_139	HEK highpH fr8		
samon_D1705_140	HEK highpH fr8		
samon_D1705_143	HEK unfr corr128kcells		
samon_D1705_144	HEK unfr corr128kcells		
samon_D1705_146	HEK unfr corr128kcells		
samon_D1705_147	HEK unfr corr128kcells		

Table S3. Samples for library generation.

Abbreviations: HSC/MPP – hematopoietic stem cell/multipotent progenitor; CMP – common myeloid progenitor; MEP – megakaryocyte-erythrocyte progenitor; GMP – granulocyte-macrophage progenitor.

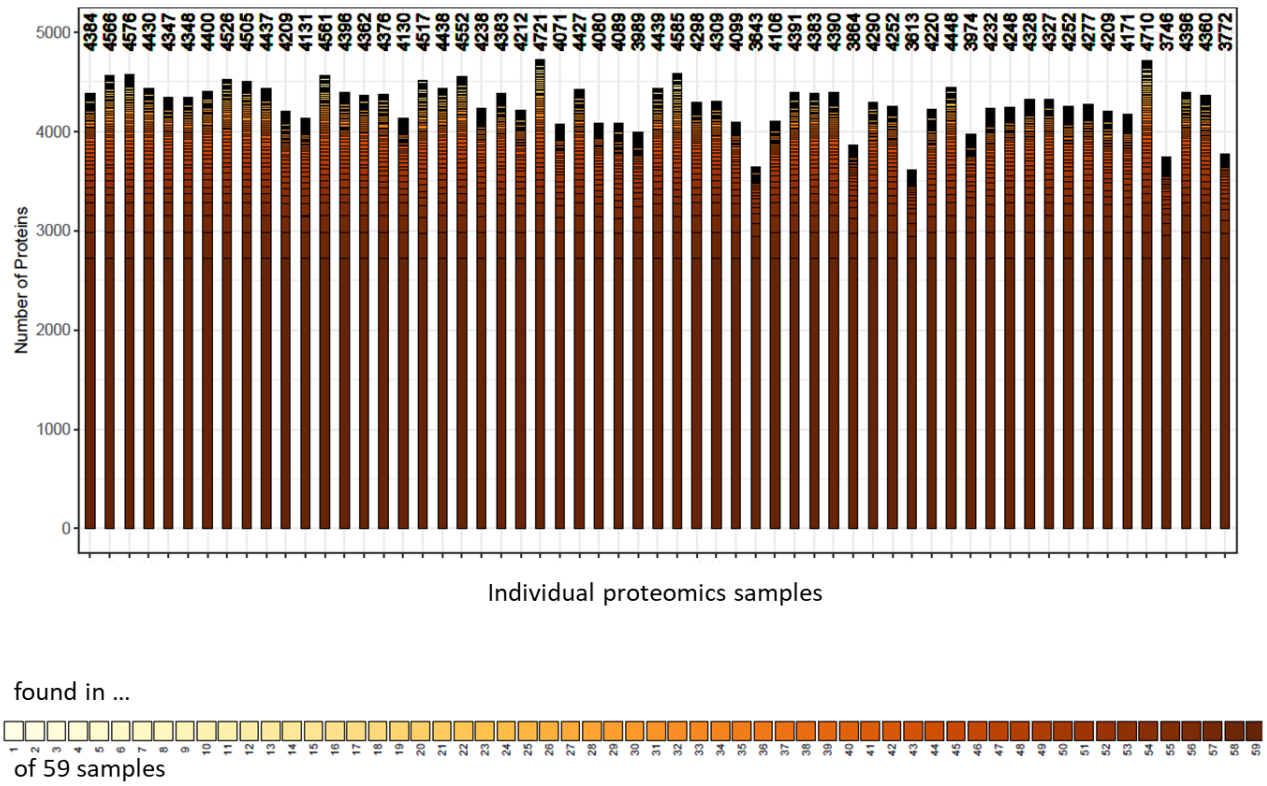


Figure S1: Number of identified proteins in individual proteomics samples.

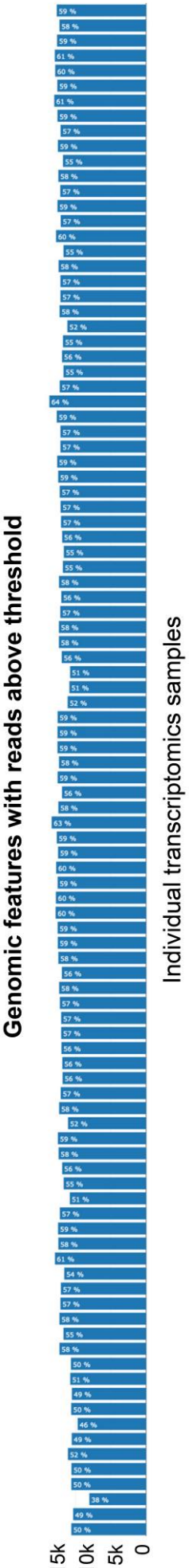
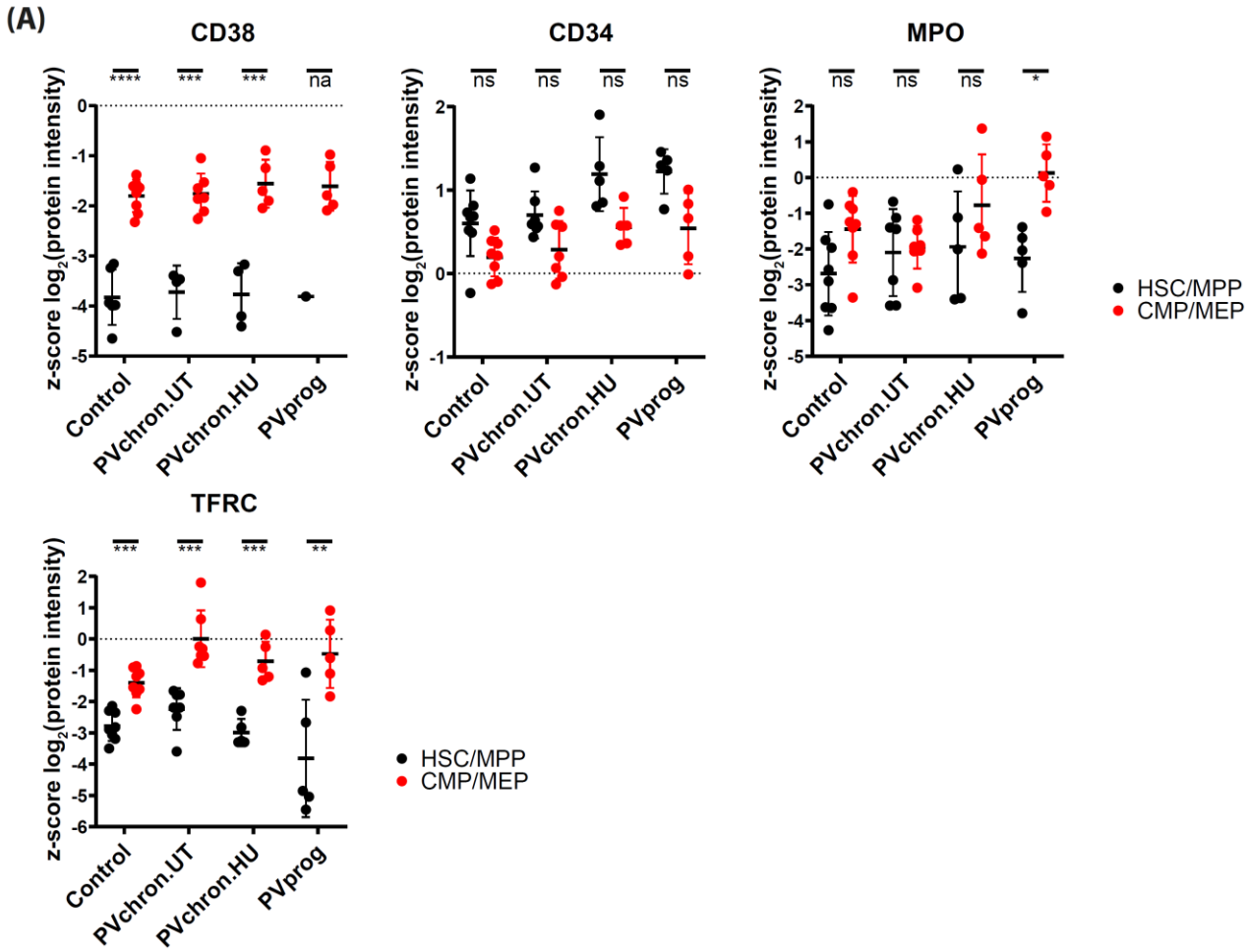


Figure S2. Number of expressed genes in individual transcriptomics samples with percentage of expressed genes over all coding genes.


(A)
CD38
CD34
MPO
TFRC
z-score log2(protein intensity)
Control
PVchron.UT
PVchron.HU
PVprog
HSC/MPP
CMP/MEP

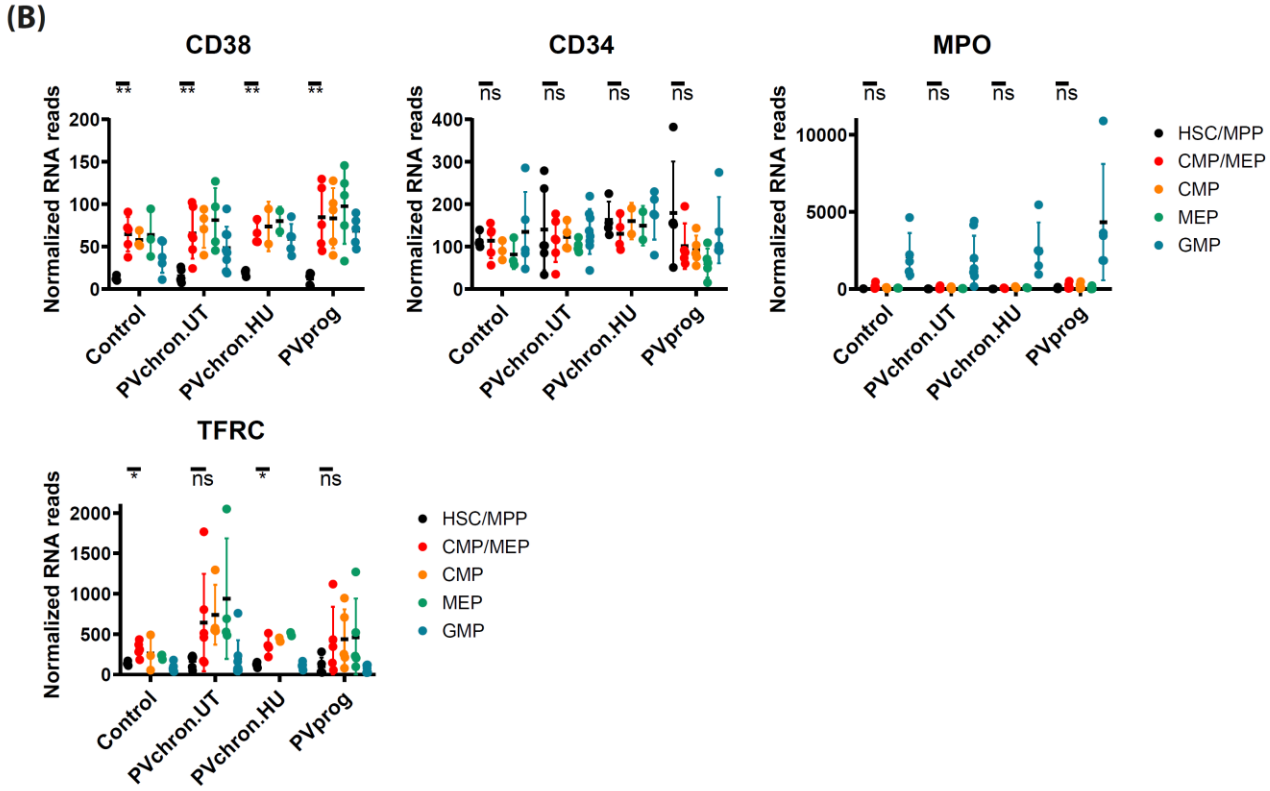

(B)
CD38
CD34
MPO
TFRC
Normalized RNA reads
Control
PVchron.UT
PVchron.HU
PVprog
HSC/MPP
CMP/MEP
CMP
MEP
GMP

Figure S3: Expression of known protein (A) and RNA (B) markers. Surface protein and lineage markers were expressed as expected and previously reported. CD38 is a surface protein marker used in the FACS-isolation of CMP/MEPs, CMPs, MEPs, GMPs ($CD38^{high}$) and HSC/MPPs ($CD38^{low}$). CD34 is the surface protein marker used for MACS-isolation and expressed in all HSPCs. MPO is a lineage marker for differentiated granulocytes and thus expected to be higher in more differentiated myeloid progenitor cells such as CMP/MEPs and especially GMPs compared to HSC/MPPs.[13] TFRC or CD71 is a lineage marker for erythroid precursors and thus expected to be higher in CMP/MEPs, CMPs and MEPs compared to HSC/MPPs.[14] NA's were not imputed. Error bars represent standard deviations. * adj. $p < 0.05$; ** adj. $p < 0.01$; *** adj. $p < 0.001$; **** adj. $p < 0.0001$ (see methods section for statistical information).
z-score log2(protein intensity) is defined as (log2(protein intensity) – mean(log2(protein intensity)))/sd(log2(protein intensity)).
Normalized RNA reads are defined as fragments per kilobase per million (FPKM), i.e. (number of fragments mapped to a gene x 10^3 x 10^6)/(total number of mapped fragments from a given library x gene length in base pairs).

Abbreviations: UT – untreated; HU – under treatment with hydroxyurea; chron – chronic; prog – progressed.

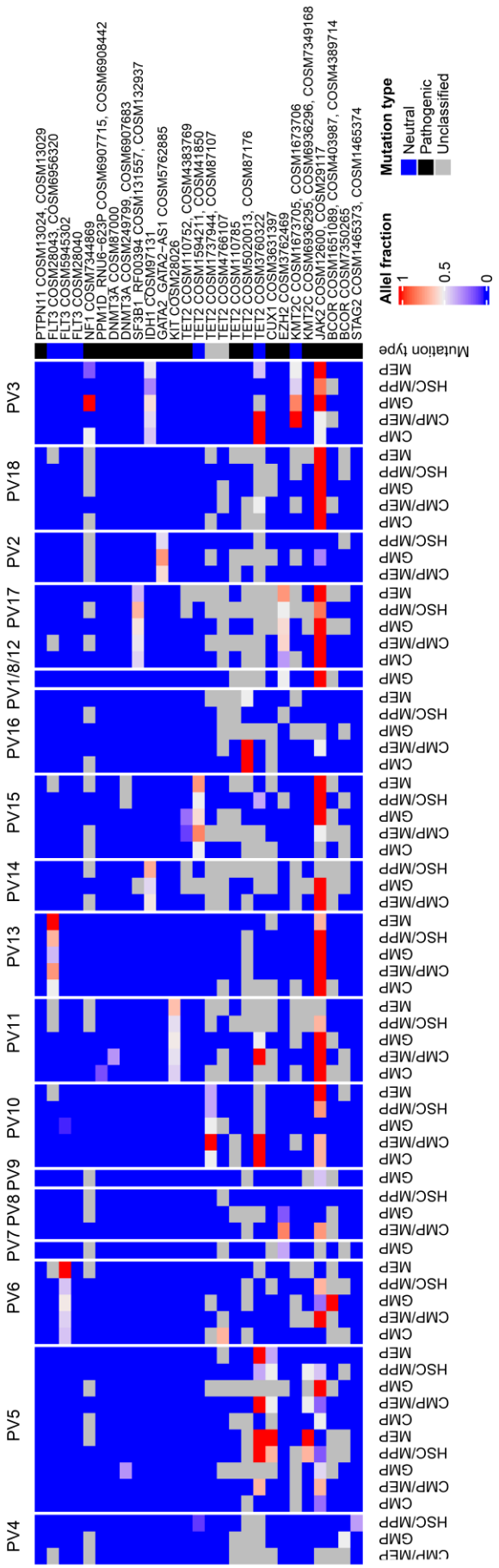


Figure S4. Mutational analysis based on RNA-seq data in patients with PV. Shown are the allele burdens of the identified mutations measured. Point mutations predicted by FATHMM as pathogenic are labelled in black, mutations classified as neutral are labelled in blue, unclassified mutations are labelled in grey.[15] See also Figure 1B and Table 1. For PV1/8/12, samples from different individuals had to be pooled to guarantee adequate HSC/MPP numbers for downstream MS and RNA-seq measurements.

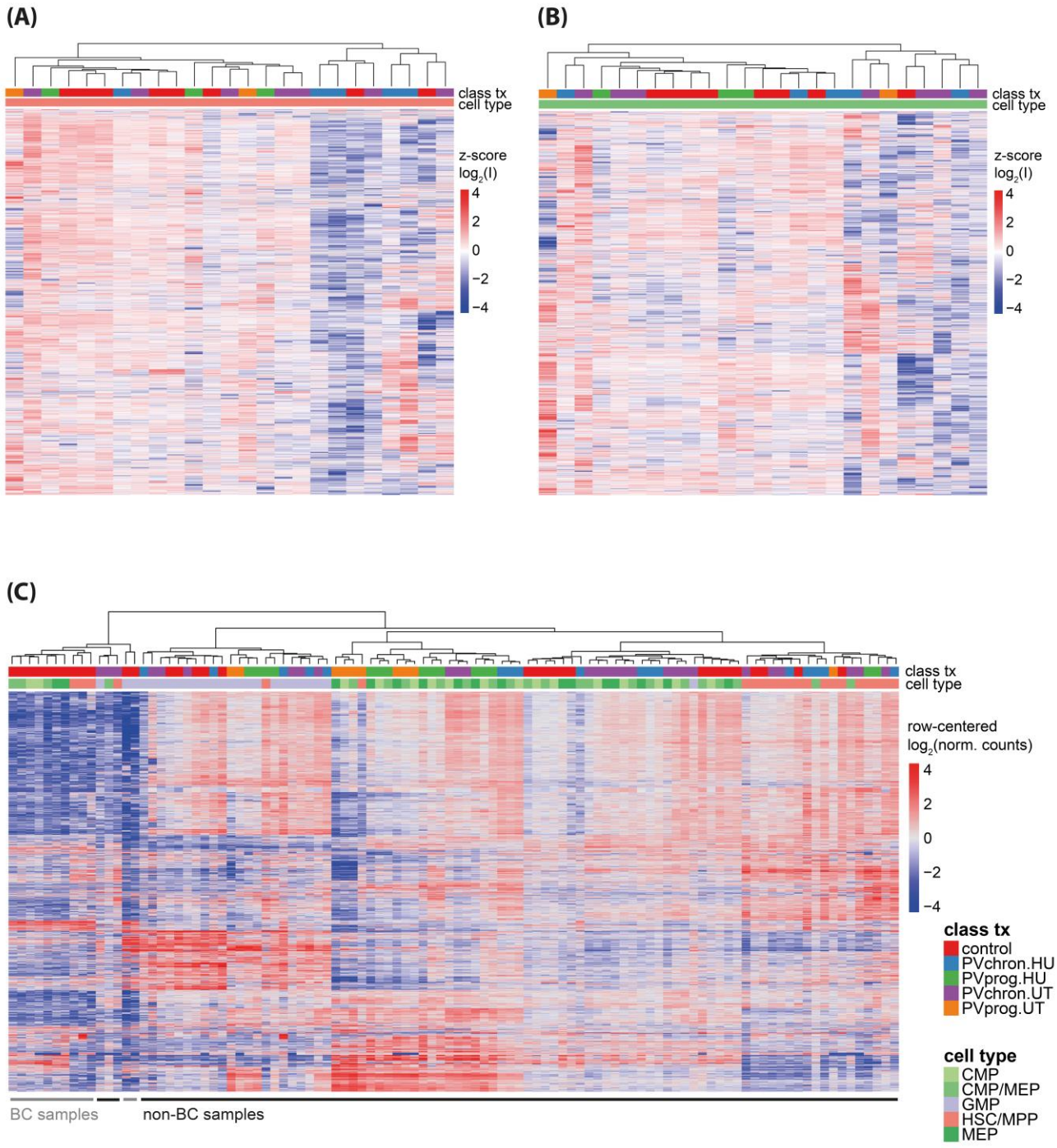


Figure S5: Heatmaps (non-supervised hierarchical complete clustering of Euclidean distances) of intensities for the 3769 proteins uniquely identified across **(A)** HSC/MPPs, **(B)** CMP/MEPs and of the **(C)** 2000 most variant mRNAs identified in HSC/MPPs, CMP/MEPs, CMPs, MEPs, and GMPs isolated from 18 PV patients and 21 controls. Z-score log2(I) refers to the z-score of log2 transformed protein intensities. Class (PV patient or control), therapy (treatment (tx) with hydroxyurea (HU) or untreated (UT)) and progression status (chronic (chron) or progressed (prog)) is marked in red, blue, green, purple and orange. In proteomic data, clustering was observed mainly according to disease stage (control vs chronic PV vs progressed PV) and to a lesser degree due to treatment (untreated vs HU-treated). In RNA-seq data, buffy coat control samples (CON1, CON2, CON3) clustered away from other samples.

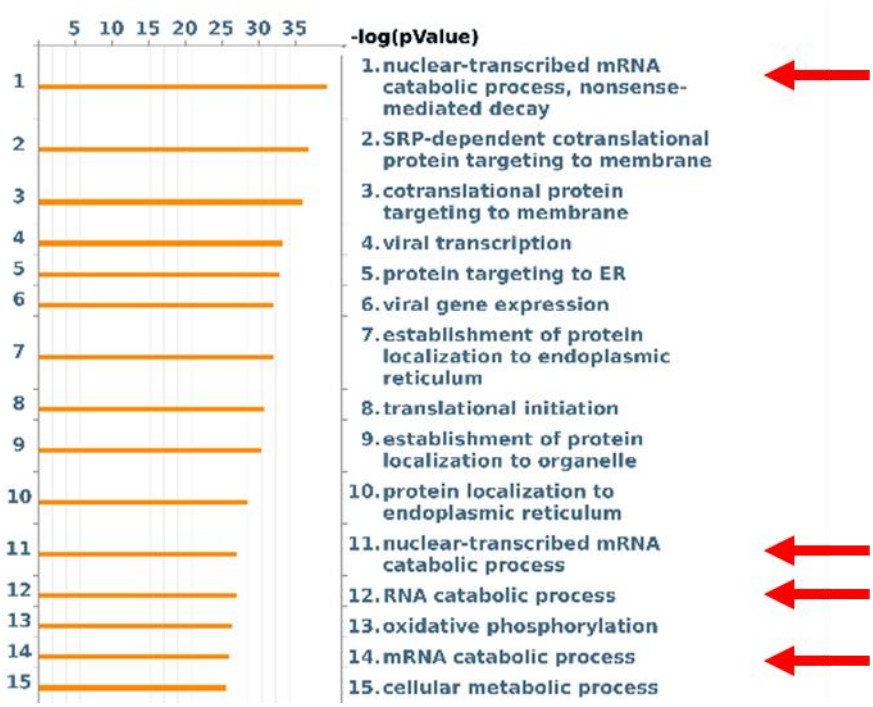
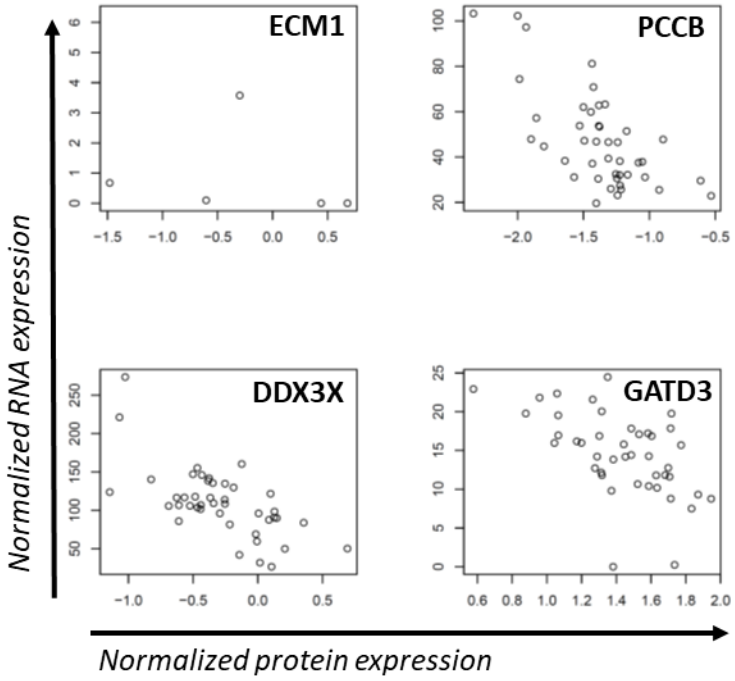


Figure S6: Metacore pathway analysis of buffy coat over non-buffy coat samples. Expression data of buffy coat samples compared to non-buffy coat samples was uploaded to Metacore (Clarivate; https://portal.genego.com; 05/2019), a web-based bioinformatics suite for pathway analysis. Pathway analysis was performed within Metacore (One-click analysis using GO Processes), applying the cutoffs of log2 FC of 1.0 and p-value of 0.05.
Buffy coat samples showed signs of RNA decay (upregulation of RNA catalytic processes). They were thus not included for further RNA analysis.

(A)



(B)

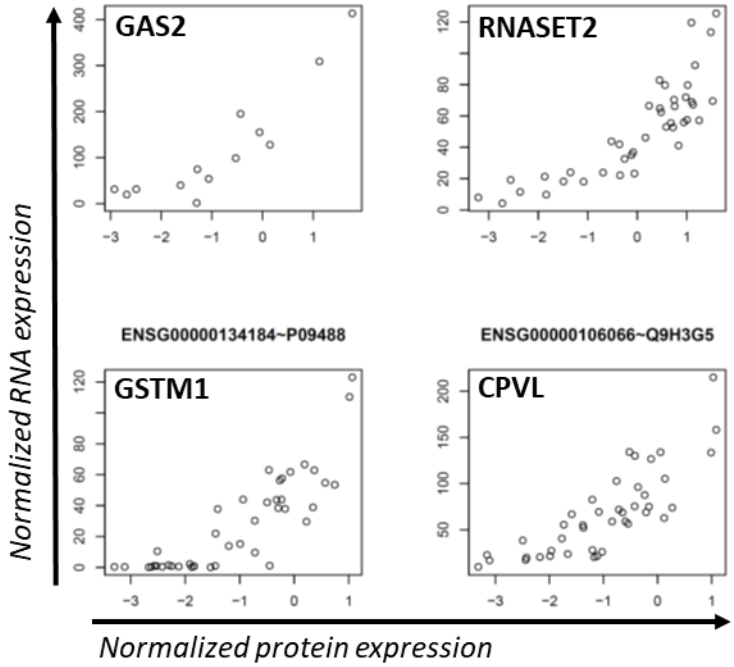


Figure S7: Correlation between protein and RNA expression. (A) Examples of negative correlation. **(B)** Examples of positive correlation.

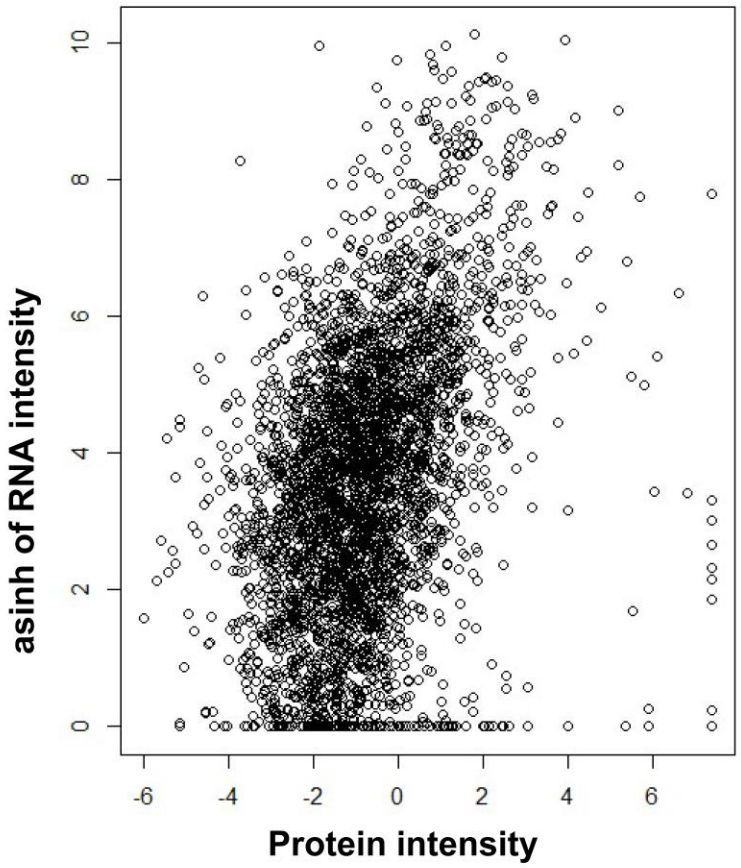
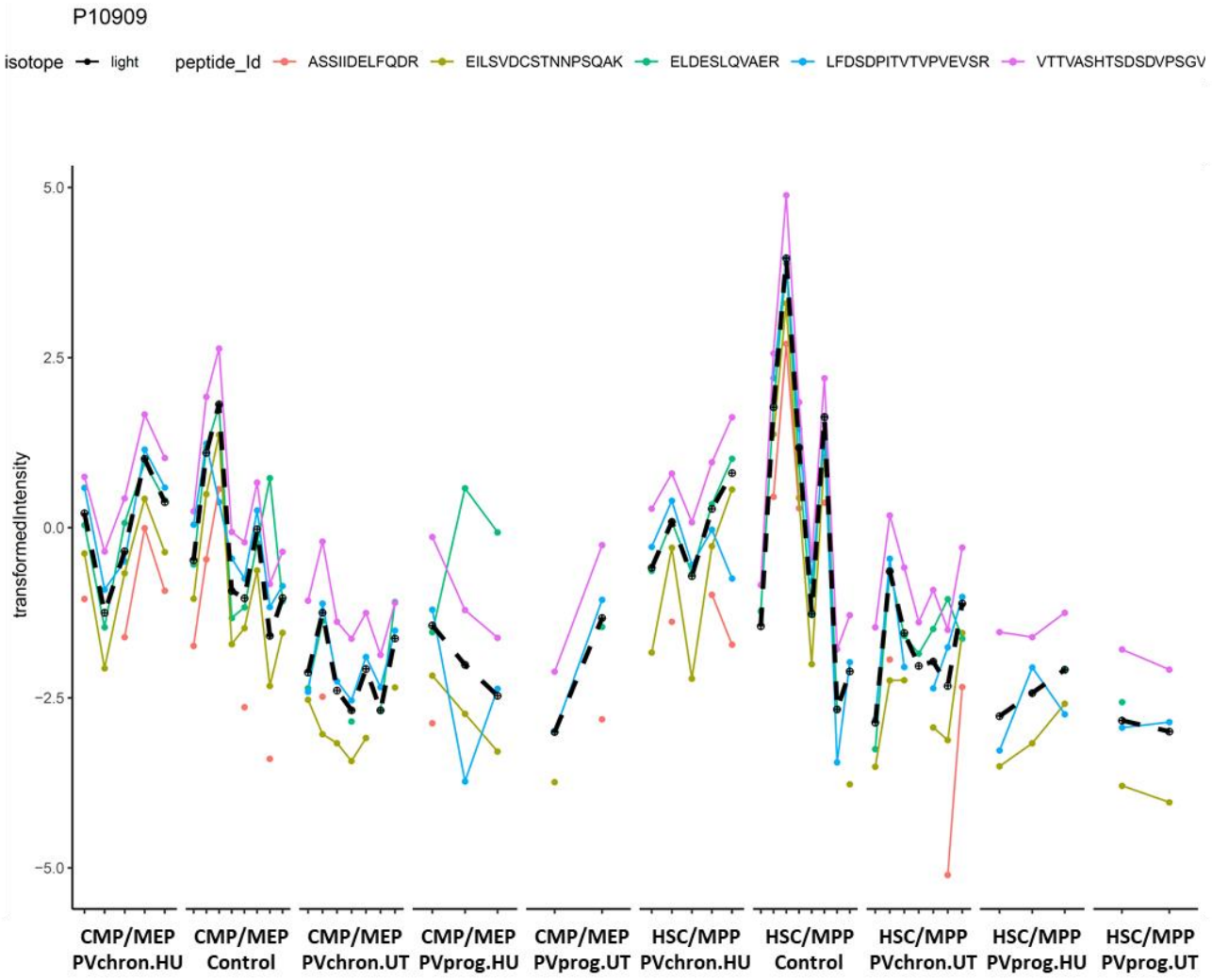
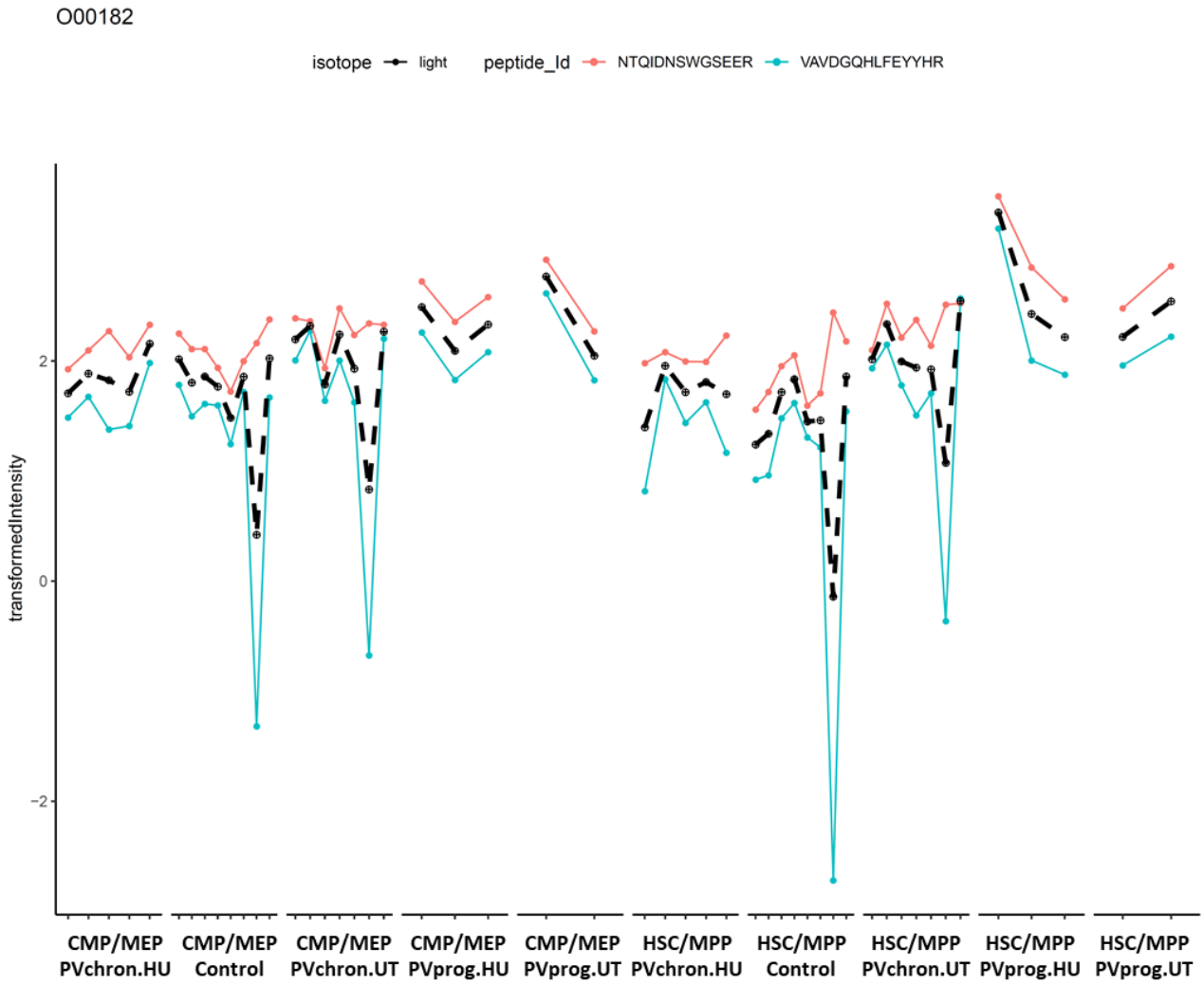


Figure S8. Correlation between protein and RNA expression intensities. Applying *cor(protMat[,1],asinh(geneMat[,1]), use="pairwise")* and *plot(protMat[,1],asinh(geneMat[,1]))* in R, a linear correlation of 0.38 was observed between RNA and protein intensity.

(A) CLU: Peptide profile as basis of CLU protein quantification



(B) LGALS9: Peptide profile as basis of LGALS9 protein quantification



(C) SOCS2: Peptide profile as basis of SOCS2 protein quantification

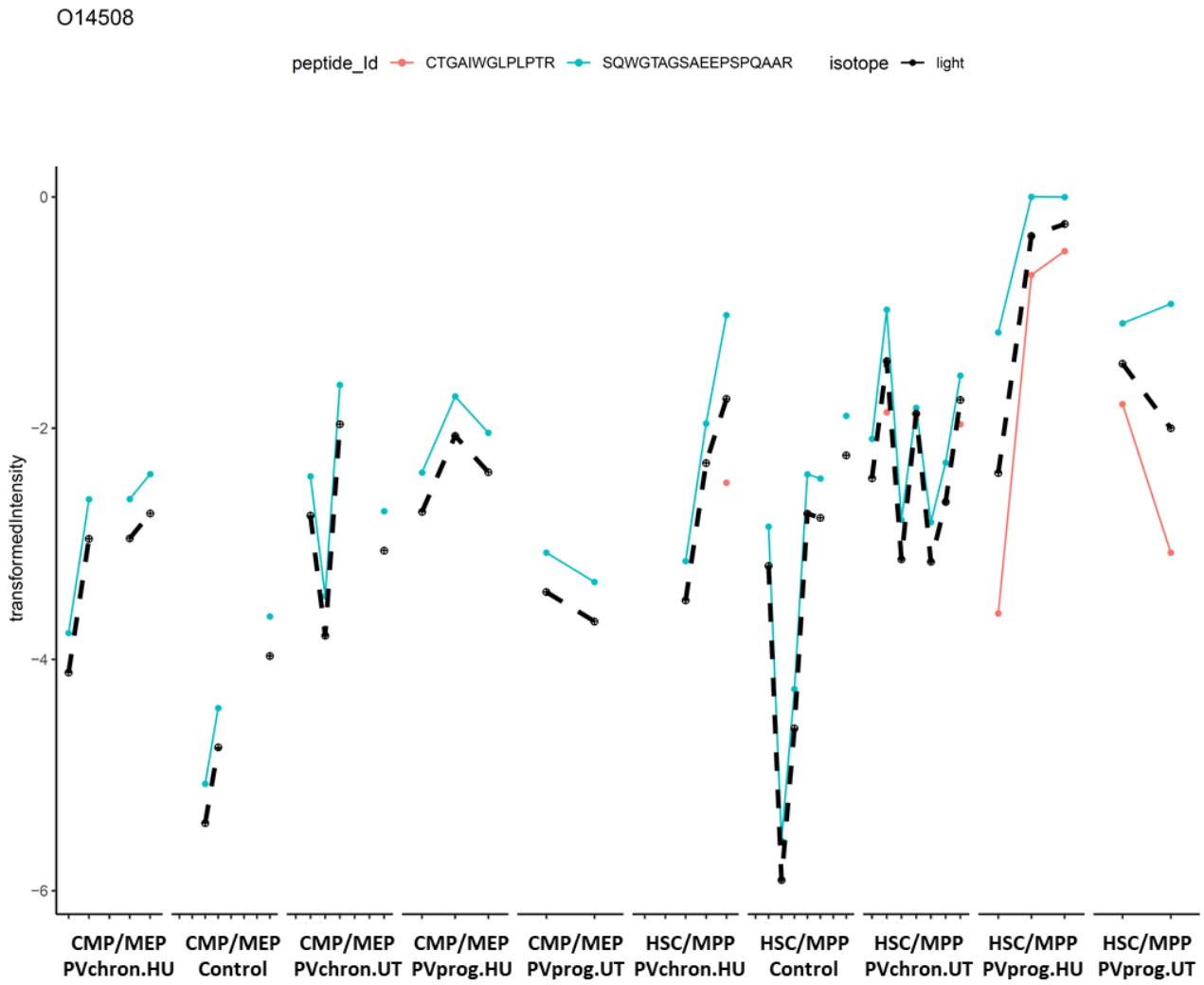


Figure S9: Transformed intensities of the identified peptides contributing to the quantification of CLU (A), LGALS9 (B) and SOCS2 (C). Individual peptides are shown in colored solid lines. Tukey's median polish is shown in dashed lines.

Abbreviations: UT – untreated; HU – under treatment with hydroxyurea; chron – chronic; prog – progressed.

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