

A novel nano-immunoassay method for quantification of proteins from CD138-purified myeloma cells: biological and clinical utility

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Supplementary methods:

Patients

CD138+ plasma cells were isolated from BM samples using the AutoMACS separation system (Miltenyi-Biotec). Fluorescence in situ hybridization (FISH) analysis for the detection of *IGH* rearrangements, 17p deletions and 1q gains was available for all patients (Supplementary Table 1). Copy number abnormalities were analyzed in 42 cases with sufficient DNA material to be able to perform CytoScan array (Affymetrix) (Supplementary Figure 1).

Myeloma cell lines

The human myeloma cell lines, NCI-H929 and U266 acquired from ATCC (American Type Culture Collection) and JLN3 from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), were used to standardize the method. Cells were routinely checked for the presence of mycoplasma with a MycoAlert kit (Lonza). Cell line identities have been tested and authenticated by short tandem repeat (STR) analysis with a PowerPlex 16 HS System kit (Promega) and online STR matching analysis (DSMZ Institute). The human STR profile database includes data sets of 2455 cell lines from ATCC, DSMZ, JCRB and RIKEN.

Capillary electrophoresis immunoassay

The immunodetection was used to quantify the abundance of each protein. In brief, 5 μ L of samples at a concentration of 0.125 mg/mL were mixed with a master mix (ProteinSimple) to a final concentration of 1x sample buffer, 1x fluorescent molecular weight markers, and 40 mM dithiothreitol (DTT), and heated at 95°C for 5 min. The samples, blocking reagent, wash buffer, primary and secondary antibodies, and chemiluminescent substrate were dispensed into designated wells in the microplate provided by the manufacturer. After plate-loading, the separation electrophoresis and immunodetection steps were carried out in the capillary system and were fully automated. Simple Western analysis was carried out at room temperature, using the default instrument settings, unless otherwise stated. The data were analyzed with the inbuilt Compass software (ProteinSimple). Each protein peak was measured automatically and normalized with respect to the GAPDH median area under the peak. Expression of each protein was presented as its abundance relative to GAPDH.

DNA/RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

DNA and RNA were extracted from samples stored in RLT Plus buffer using an Allprep DNA/RNA kit (Qiagen) following the manufacturer's protocol. Total RNA (400 ng) was reverse-transcribed to cDNA using a high-capacity cDNA reverse-transcription kit.

Statistical analysis

The percentage coefficients of variation (CVs) of the performance of each antibody and the mRNAs and proteins measured in the analyzed population of MM were calculated. The statistical similarity between mRNA and protein expression variability was estimated using the Feltz and Milles test for CVs of k populations and by Krishnamoorthy's and Lee's modified signed-likelihood ratio test (SLRT) for equality of CVs, using the cvequality package (version 0.1.1) in R ^{1,2}. Spearman correlations were also calculated.

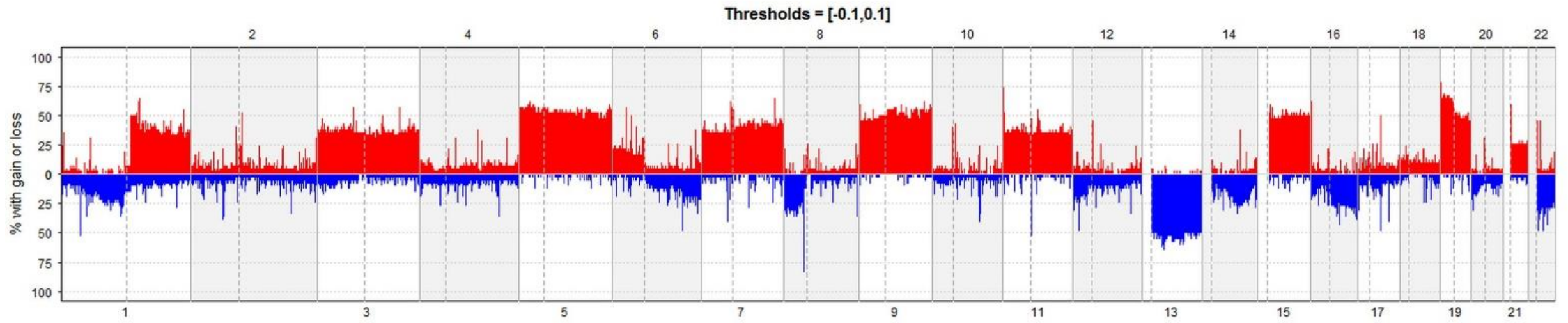
Cutoff Finder software (<http://molpath.charite.de/cutoff>) was used to determine the optimal cutoff, which was defined as that providing the most significant split that discriminated between long and short survival when testing all the possible cutoffs using the log-rank test ³.

References:

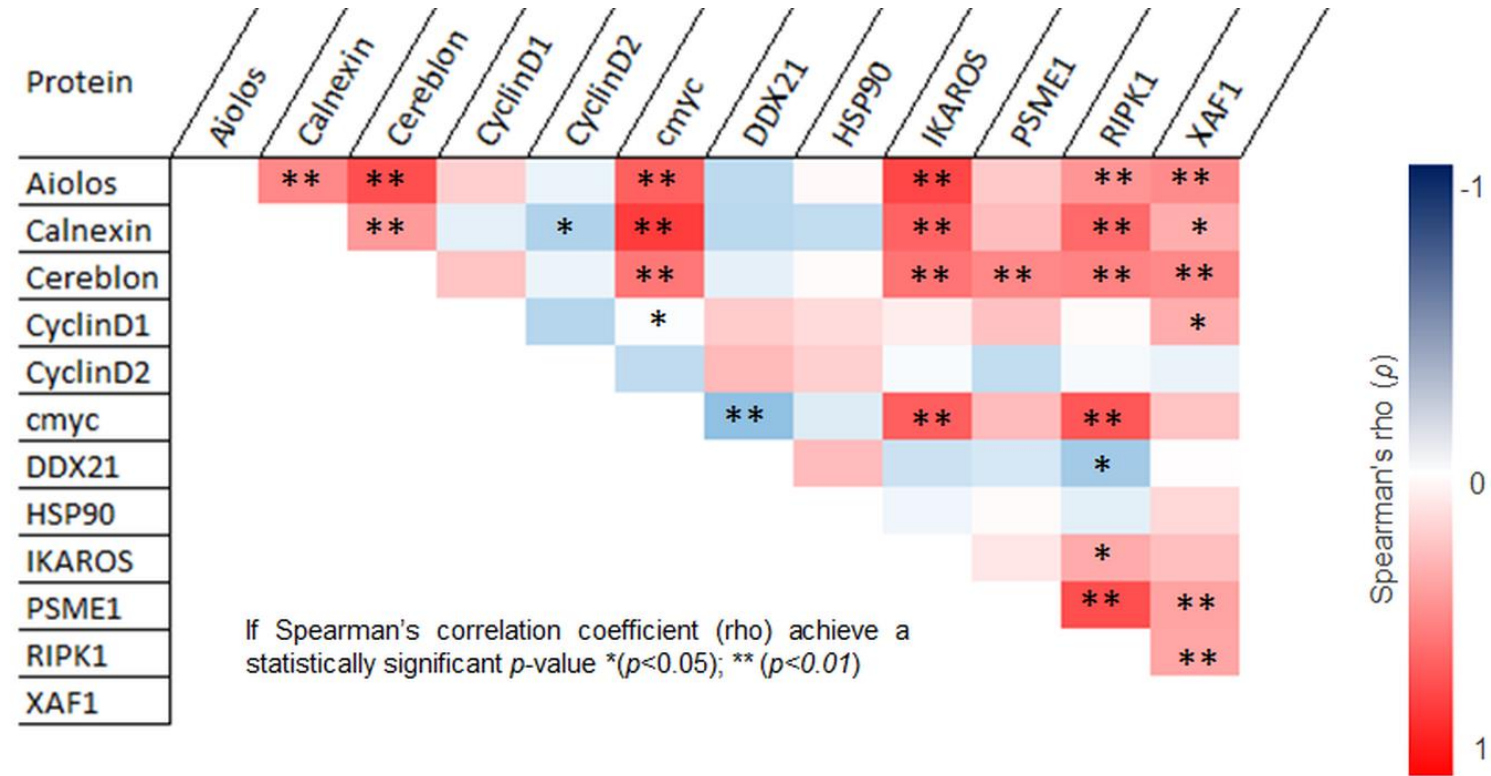
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Supplementary Table 1. Clinical and biological characteristics of MM patients

	<i>Samples included in the study n=63</i>	<i>Samples included in the survival analysis n=43</i>
<i>Age (years), median (range)</i>	69.2 (39.6-87.6)	67.7 (39.6-87.6)
<i>Sex (male/female)</i>	37/26	27/16
<i>International staging system n (%)</i>		
I	14 (22.2%)	9 (20.9%)
II	24 (38.1%)	22 (51.1%)
III	14 (22.2%)	10 (23.5%)
Not determined	11 (17.5%)	2 (4.5%)
<i>Cytogenetic characteristics n (%)</i>		
1q gain	28 (44.4%)	20 (46.5%)
17p del	6 (9.5%)	3 (6.9%)
t(4;14)	7 (11.1%)	5 (11.6%)

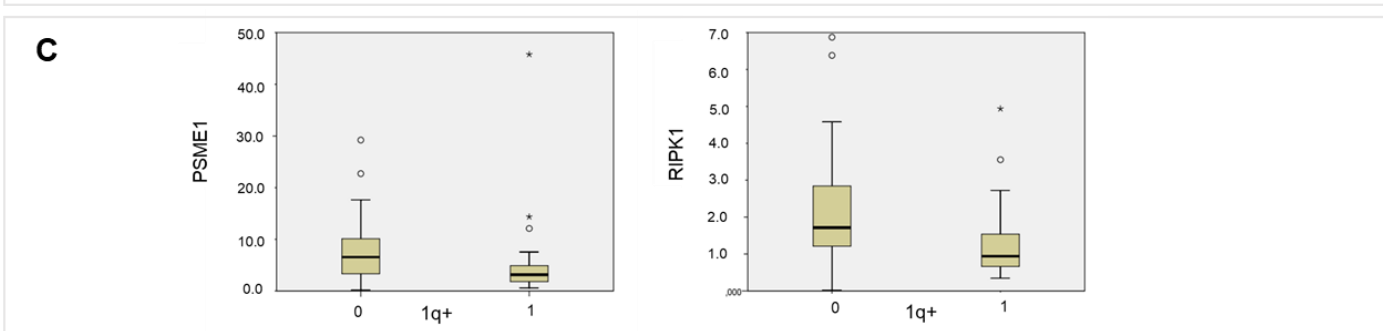
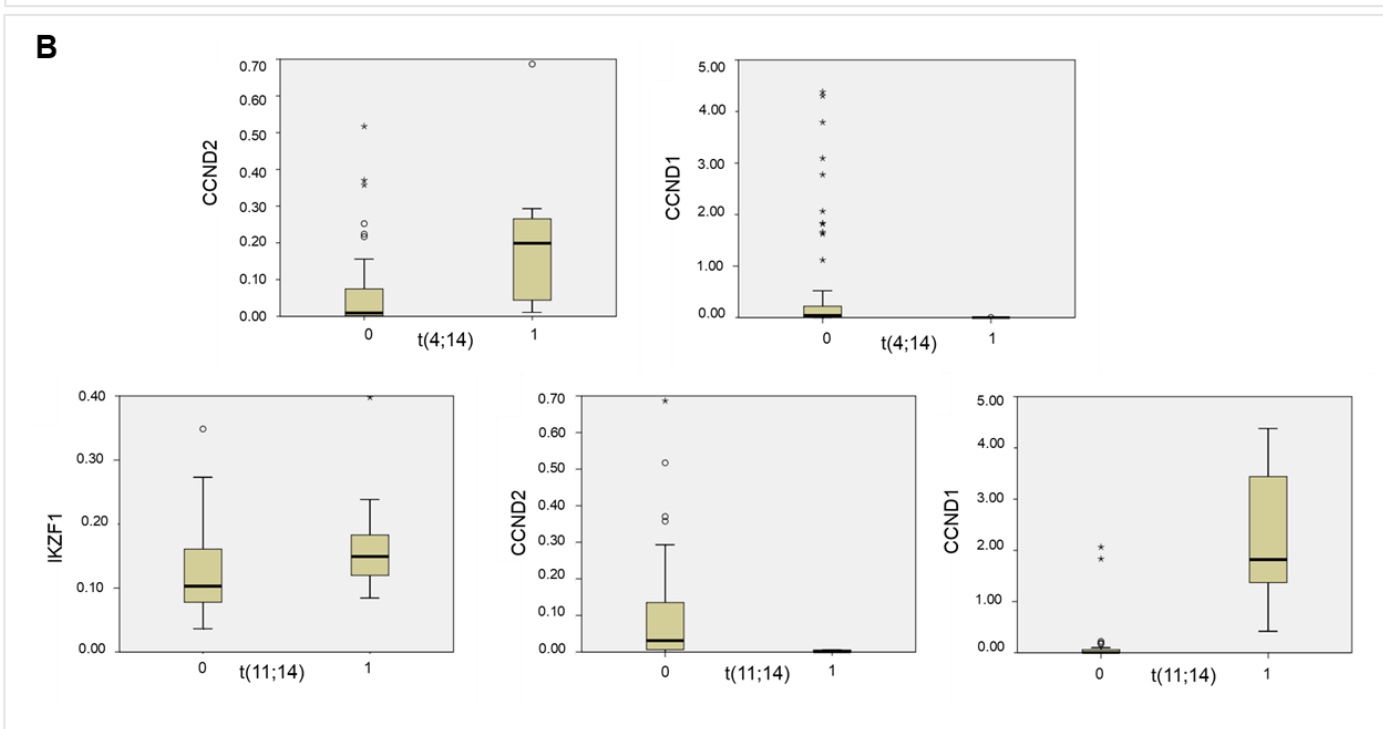
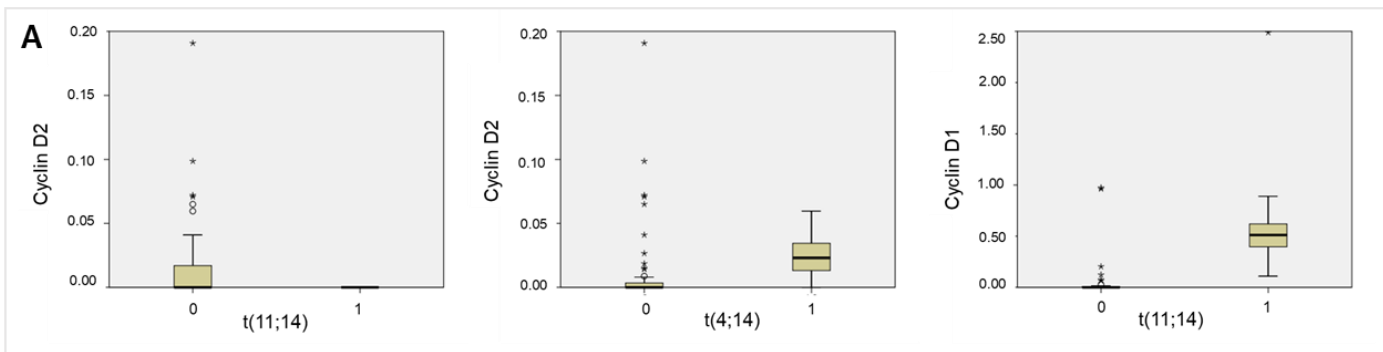


Supplementary Figure 1. Frequency plot of copy number changes at a genomic position in MM sample (n=42)



Supplementary Figure 2. Correlation between levels of expression of each protein pair in MM samples.

Heat map showing univariate analysis of protein abundance, considering statistically significant Spearman correlations (*, $p < 0.05$; **, $p < 0.01$).



Supplementary Figure 3.

(A) Level of expression of cyclin D1 and D2 proteins in the presence or absence of the indicated cytogenetic abnormality detected by FISH analysis.

(B) Level of expression of IKZF_1, CCND1 and CCND2 in the presence or absence of the indicated cytogenetic abnormality detected by FISH analysis.

(C) Level of PSME1 and RIPK1 proteins in the presence or absence of 1q gain.