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SUPPLEMENTAL METHODS

CSC-technology

Approximately 100 million cells from each biological replicate (n=3-6) were taken through the CSC-Technology workflow as previously described in detail.(1-3) Cells were washed with PBS and oxidized by treatment with 1 mM sodium meta-periodate (Pierce, Rockford, IL) in PBS pH 7.6 for 15 min at 4°C followed by 2.5 mg/ml biocytin hydrazide (Biotium, Hayward, CA) in PBS pH 6.5 for 1 hour at 4°C. Cells were then collected and homogenized in 10mM Tris pH 7.5, 0.5 mM $MgCl_2$ and the resulting cell lysate was centrifuged at 800 x g for 10 min at 4°C. The supernatant was centrifuged at 210,000 x g for 16 hours at 4°C to collect the membranes. The supernatant was removed and the membrane protein pellet was washed with 25 mM Na_2CO_3 to disrupt peripheral protein interactions. To the resulting membrane pellet, 300µl 100 mM NH_4HCO_3, 5 mM Tris(2-carboxyethyl) phosphine (Sigma, St. Louis, MO), and 0.1% (v/v) Rapigest (Waters, Milford, MA) were added and placed on a Thermomixer (750 rpm) to continuously vortex. Proteins were allowed to reduce for 10 min at 25°C followed by alklylation with 10 mM iodoacetamide for 30 min. The sample was incubated with 20 µg proteomics grade trypsin (Promega, Madison, WI) at 37°C overnight. Samples were acidified with 5 µl phosphoric acid (88%) then centrifuged at 14,000 rpm for 10 min to remove particulates. The resulting peptide mixture was incubated with 450 µl bead slurry of UltraLink Immobilized Streptavidin PLUS (Pierce, Rockford, IL) for 1 hour at 25°C. Beads were sequentially washed with 10mL each of 0.05% Triton X-100 in 100 mM NH_4HCO_3, 5M NaCl, 100 mM NH_4HCO_3, 100 mM Na_2CO_3, and 80% isopropanol to remove non-specific peptides and lipids. Beads were resuspended in 100 mM NH_4HCO_3 and 500 units glycerol-free endoproteinase PNGaseF (New England Biolabs, Ipswich, MA) and incubated at 37°C overnight

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with end-over-end rotation to release the peptides from the beads. Collected peptides were desalted and concentrated using a C_{18} MicroSpin™ column (Harvard Apparatus, Holliston, MA) according to manufacturer's instructions.

Preparation of whole cell lysates for PRM assays

Cell pellets (approx. 10×10^6 cells per pellet) were resuspended in 240 µL 100 mM ammonium bicarbonate, 120 µL acetonitrile, and 240 µL Invitrosol LC/MS Protein Solubilizer (5X solution, Thermo Scientific) for 600 µL total lysis buffer volume. This total volume was scaled in equal parts based on cell count, to 200 µL for pellets with 0.8×10^6 – 3.7×10^6 cells or to 1200 µL for pellets with 20×10^6 cells. Downstream additions of TCEP, iodoacetamide, trypsin, and 10% trifluoroacetic acid (TFA) were also scaled accordingly. Cell suspensions were sonicated (VialTweeter, Hielscher, Teltow, Germany) in microcentrifuge tubes using a 10 s sonication pulse followed by a 10 s pause on ice, with this cycle repeated 10 times total. 33 µL of 100 mM TCEP was added to each tube. Tubes were vortexed then incubated with shaking at 37 °C and 1400 rpm for 30 minutes. 66 µL 100 mM iodoacetamide was added to each tube and tubes were incubated with shaking for another 30 minutes in the dark. 20 µg sequencing grade trypsin (Promega) was added to each tube and digestion proceeded at 37 °C and 1400 rpm overnight. 30 µL 10% TFA was added to each tube to quench digestion. In both cell line and patient sample analyses, a 20 µL aliquot was taken from each sample and cleaned using SP2(4) and eluted into 100 µL 2% acetonitrile 98% water. Peptide concentrations were determined using Pierce Quantitative Fluorometric Peptide Assay (Thermo). Cleaned samples were then diluted with 2% acetonitrile, 98% water with 0.1% formic acid to a final working concentration of 25 ng/µL total sample peptide concentration with Pierce Peptide Retention Time Calibration

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Mixture (PRTC, Thermo) spiked in, to a final concentration of 1 fmol/µL PRTC. Chromatography, MS and data analysis details are outlined in Supplemental Tables 11 and 12, below.

PRM assay development

Multiple myeloma cell lines were digested and first analyzed by data dependent acquisition to inform peptide target selection. Three replicate injections each of 500 ng total peptide were analyzed by the method outlined in the table below. MS data were analyzed using Proteome Discoverer 2.2 (Thermo) platform as outlined in the table below. Identified peptides belonging to proteins of interest (selected from CSC-Technology and intracellular controls) were used to generate a peptide precursor ion target list for follow up parallel reaction monitoring (PRM) analyses. Selection criteria included: at least 6 amino acids in length, unique to a master protein accession (*i.e.* not counting isoforms), and produced at least 5 identified fragment ions in the MS^2 spectrum. Peptides containing missed tryptic cleavage sites or methionine oxidation were excluded. A maximum of 7 peptides were targeted per protein. All PRM data were analyzed using Skyline (5) software with spectral libraries generated from PD2.2 search results of the DDA data. Chromatographic peak areas are defined by sum of MS^2 fragment ion signal for identified fragments within a manually surveyed chromatographic peak. Five variations of the normalized collision energy (NCE; 25, 27, 28, 29, or 31) were tested for all peptide targets. For NCE optimization, peptides from all six cell lines were pooled in equal parts and analyzed as two technical injections of 1000 ng total peptide per method. Following analysis in Skyline, the final method was designed to use NCE values that produced the greatest chromatographic peak area for total MS^2 fragment ion signals.

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PRM assay application

Whole cell lysate digestions of the six cell lines (n=3 biological replicates) and six multiple myeloma patients (CD138+ and CD138- fractions) were prepared as described above. For all samples, 500 ng total peptide injections were analyzed in technical triplicate. Samples were first analyzed using a data-dependent acquisition method applied to tryptic digests of whole cell lysates to enable selection of unmodified peptides (*i.e.* not glycopeptides) from cell surface proteins as well as several intracellular proteins of interest. Fully tryptic peptides were selected for parallel reaction monitoring (PRM)(6) assay development and chromatography and normalized collision energies were optimized to obtain at least 10 points across the peak and maximum fragment ion intensity. Skyline(5) was used for all analyses. Overall, suitable results were obtained for 209 peptides from 73 proteins, including 48 cell surface proteins and 14 intracellular controls.

These PRM assays were applied to the same 6 cell lines (n=3 biological replicates each) used for discovery by CSC. To guard against system bias, pooled quality control (Pooled QC) samples were generated by combining equivalent peptides from each of the 6 cell lines per biological replicate and analyzed prior to and after each replicate sample block. Pooled QC samples were generated from respective biological replicate batches. Technical replicate blocks were queued in uniquely randomized order per block. Each block was preceded and followed by analysis of the pooled QC sample. This block/randomization was repeated for each biological replicate. For the primary cells, the six patient samples were blocked by technical replicate, with each fraction analyzed in alternating blocks. Pooled QC samples for each fraction (CD138+, CD138-) were prepared separately and analyzed prior to their respective blocks. A final pooled

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QC run for CD138+ followed immediately by CD138- was inserted at the end of the overall sample queue. For all PRM analyses, Pierce Peptide Retention Time Calibration Mixture (PRTC, Thermo) was spiked in, to a final concentration of 1 fmol/µL and 500 ng total peptide injections were analyzed in triplicate per sample. Chromatography and MS parameters are described in the Supplemental Table 13, below.

Data were imported into Skyline and chromatographic peaks were extracted from MS^2 spectra for each detected peptide from the target list. The mean total fragment ion peak areas of the six patients' CD138+ and CD138– samples were compared using a parametric ratio paired t-test using GraphPad Prism. Statistical significance is assigned by p-value <0.05. On graphs, p-value represented by annotations: n.s. for $p > 0.05$, * for $p<0.05$, ** for $p<0.01$, *** for $p<0.001$, **** for $p<0.0001$.

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Supplemental Table 1: Exported peptide lists (formatted as an excel file).

Supplemental Table 2: List of proteins identified by CSC (formatted as an excel file).

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Supplemental Table 3: Patient sample characteristics

	Cells recovered	% Plasma in BM	% Purity	Phenotype
Cohort 1				
Patient 10	$48.0x10^6$	12.6	98.9	No data
Patient 11	$5.0x10^6$	2.0	86.9	CD56-, CD19-
Patient 12	$41.4x10^6$	51.2	83.8	CD56-, CD19-
Patient 13	$2.1x10^6$	52.6	89.0	CD56-, CD19-
Patient 14	$1.0x10^6$	20.8	23.3	CD56+, CD19-
Patient 15	$2.3x10^6$	20.8	32.8	CD56+, CD19-
Cohort 2				
Patient 16	$35.0x10^6$	32.2	92.5	CD56-, CD19-
Patient 17	$2.3x10^6$	2.2	27.8	CD56-, CD19+
Patient 18	$15.4x10^6$	48.4	84.5	CD56-, CD19-
Patient 19	$26.3x10^6$	24.4	92.4	CD56+, CD19-

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332 **Supplemental Table 4**: Cell surface *N*-glycoproteins that are known MM antigens and were

333 selected for detection by PRM.

Protein	Description
CD19	A B cell marker not typically considered to be a therapeutic target for MM, however it may be expressed on a minor MM stem cell subset(7) CD19 CARs have been used to treat MM even in the absence of CD19 detection on 99.95% of MM cells(8) In our study, CD19 was not detected by M/S on the CD138- or CD138+ samples
CD20	A B cell marker, CD20 expression on MM has been reported in 18% of MM patients(9) In our study, CD20 was identified at very low levels across our patient samples, with the exception of one patient with high CD20 expression in the CD138+ subset
CD27	No PRM assay developed
CD28	A co-stimulatory protein important for T cell activation, aberrant CD28 expression has been reported on MM cells from 41% of myeloma patients(10)
CD33	No PRM assay developed
CD38	Confirmed to have significantly higher expression on the isolated MM cells, supporting the validity of our approach
CD44	An adhesion molecule with roles in migration and homing, CD44 expression levels have been associated with MM progression(11-13) No significant difference in CD44 expression was present overall, however one patient in this study had much higher expression of CD44 on their CD138+ cell subset
CD45	Expressed on all nucleated hematopoietic cells, MM cells are reported to have two distinct populations with low and high CD45 expression(14, 15) CD45 expression was identified here as significantly lower on the CD138+ cell population, however expression was still detected at some level in cells from all patients, consistent with previous reports(16-18)
CD52	No PRM assay developed
CD54	Associated with advanced disease and drug resistance in MM(19-21) Identified here as having significantly higher expression in CD138+ cells An anti-CD54 mAb has already completed phase II trials for smoldering MM(22)
CD56	Expression is known to be variable on MM cells and may have prognostic significance (23-25) In our study, two of five evaluated patient samples were CD56+ by flow cytometry, however a PRM assay for CD56 was not successfully developed
CD177	No PRM assay developed
CD138	Confirmed to have significantly higher expression on the isolated MM cells, supporting the validity of our approach
CD200	No PRM assay developed

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BCMA	A plasma cell marker, and already an immunotherapy target for MM(26, 27) Significant differences in BCMA expression between CD138+ and CD138- samples were not found in this patient set

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335 **Supplemental Table 5**: Cell surface *N*-glycoproteins with significantly higher abundance in the

336 CD138+ MM patient cell subset by PRM analysis.

Uniprot ID	Description	CD protein	Significance
O43852	Calumenin (CALU)	---	***
O60449	Lymphocyte antigen 75 (LY75)	CD205	*
P00734	Prothrombin (F2)	---	**
P01859	Immunoglobulin heavy constant gamma 2 (IGHG2)	---	**
P02787	Serotransferrin (TF)	---	*
P04180	Phosphatidylcholine-sterol acyltransferase (LCAT)	---	**
P04216	Thy-1 membrane glycoprotein	CD90	**
P05026	Sodium/potassium-transporting ATPase subunit beta-1 (ATP1B1)	---	**
P05362	Intercellular adhesion molecule 1 (ICAM1)	CD54	***
P06127	T-cell surface glycoprotein CD5	CD5	**
P08195	4F2 cell-surface antigen heavy chain	CD98hc	**
P13598	Intercellular adhesion molecule 2 (ICAM2)	---	***
P16615	Sarcoplasmic/ endoplasmic reticulum calcium ATPase 2 (SERCA2, ATP2A2)	---	***
P18827	Syndecan-1 (SDC1)	CD138	****
P20020	Plasma membrane calcium-transporting ATPase 1 (ATP2B1, PMCA1)	---	*
P20226	TATA-box-binding protein (TBP)	---	**
P21796	Voltage-dependent anion-selective channel protein 1 (VDAC1)	---	**
P26010	Integrin beta-7 (ITGB7, LPAM-1)	---	**
P28907	ADP-ribosyl cyclase/ cyclic ADP-ribose hydrolase 1	CD38	****
P35613	Basigin (EMMPRIN, BSG)	CD147	***
P43307	Translocon-associated protein subunit alpha (SSR1, TRAP-alpha)	---	***
P50851	Lipopolysaccharide-responsive and beige-like anchor protein (LRBA, CDC4L)	---	***
P52799	Ephrin-B2 (EFNB2)	---	***
Q00059	Transcription factor A, mitochondrial (TFAM, mtTFA)	---	**
Q13740	CD166 antigen (ALCAM, MEMD, CD6L)	CD166	**
Q8IYS5	Osteoclast-associated immunoglobulin-like receptor (OSCAR)	---	*
Q96KA5	Cleft lip and palate transmembrane protein 1-like protein (CLPTM1L)	---	****
Q9NSC5	Homer protein homolog 3 (Homer3)	---	**
Q9NZK5	Adenosine deaminase 2 (ADA2)	---	**
Q9UJJ9	N-acetylglucosamine-1-phosphotransferase subunit gamma (GNPTG)	---	*

337 * for $p<0.05$, ** for $p<0.01$, *** for $p<0.001$, **** for $p<0.0001$

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338 **Supplemental Table 6**: Cell surface *N*-glycoproteins identified by PRM on primary MM cells

339 with potential diagnostic or prognostic significance.

Protein	Prognostic Significance	Reference
CD90	Associated with tumorigenesis and poor survival in hepatocellular carcinoma, hepatoblastoma, and lung cancer	(28-32)
ATP1B1	In cytogenetically normal AML this protein is associated with shorter overall survival	(33)
CD5	Identified as a poor prognostic marker for cancers such as diffuse large B-cell lymphoma and mantle cell lymphoma	(34, 35)
CD98hc	Associated with poor survival in cancers such as oropharyngeal cancer, hypopharyngeal squamous cell carcinoma, and gastric cancer	(36-38)
ICAM2	Increased expression has been associated with poor survival in various cancers May be associated with anti-tumor immune response	(39, 40)
SERCA2 (ATP2A2)	High expression has been associated with tumor grade and metastasis in colorectal cancer Expression has been associated with response to bortezomib in liposarcoma	(41, 42)
TBP	Known to be upregulated by oncogenic signaling pathways, and may play an early role in tumorigenesis of cancers such as colon carcinomas and adenomas	(43)
LRBA	Shown to predict for mortality and recurrence in breast cancer	(44)
EFNB2	Significant correlations between expression, overall survival, and disease-free survival have been noted in various solid tumors	(45-48)
HOMER3	Overexpression is significantly associated with advanced stage in esophageal squamous cell carcinoma	(49)
ADA2	Increased expression has been correlated with lymph node involvement, grade, and tumor size in breast cancer	(50)
CLPTM1L	Overexpression has been associated with poor prognosis in lung cancer Demonstrated to play a role in cisplatin resistance	(51, 52)
CD166	Plays a significant role in MM progression and BM homing Strongly correlated with unfavorable prognosis in melanoma	(53, 54)

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341 **Supplemental Table 7**: Proteins with no significant difference in abundance in the CD138+ cell

342 subset as determined by PRM

Uniprot ID	Description	CD protein	Detected by PRM?
O60266	Adenylate cyclase type 3 (ADCY3)	---	Yes
P01033	Metalloproteinase inhibitor 1 (TIMP1)	---	Yes
P02786	Transferrin receptor protein 1 (TFR1)	CD71	Yes
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1)	---	Yes
P11836	B-lymphocyte antigen CD20	CD20	Yes
P12259	Coagulation factor V (F5)	---	Yes
P16070	CD44 antigen	CD44	Yes
P23634	Plasma membrane calcium-transporting ATPase 4 (ATP2B4)	---	Yes
P32942	Intercellular adhesion molecule 3 (ICAM3)	---	Yes
P54709	Sodium/ potassium-transporting ATPase subunit beta-3 (ATP1B3)	---	Yes
P55083	Microfibril-associated glycoprotein 4 (MFAP4)	---	Yes
P84243	Histone H3.3 (H3F3A)	---	Yes
Q02223	Tumor necrosis factor receptor superfamily member 17 (BCMA, TNFRSF17)	---	Yes
Q13510	Acid ceramidase (ASAH1)	---	Yes
Q14242	P-selectin glycoprotein ligand 1 (SELPLG)	---	Yes
Q8NBM8	Prenylcysteine oxidase-like (PCYOX1L)	---	Yes
Q92626	Peroxidasin homolog (PXDN)	---	Yes
Q96G23	Ceramide synthase 2 (CERS2)	---	Yes
Q99523	Sortilin (SORT1)	---	Yes
Q9H813	Transmembrane protein 206 (TMEM206)	---	Yes
Q9NZT1	Calmodulin-like protein 5 (CALML5)	---	Yes
Q9UBK2	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A)	---	Yes
Q9Y6X5	Bis(5'-adenosyl)-triphosphatase ENPP4	---	Yes

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Supplemental Table 8: Proteins with significantly lower abundance in the CD138+ cell subset as determined by PRM.

Uniprot ID	Description	CD protein	Detected by PRM?
P04234	T-cell surface glycoprotein CD3 delta chain	CD3D	Yes
P05107	Integrin beta-2 (ITGB2)	CD18	Yes
P08575	Receptor-type tyrosine-protein phosphatase C (PTPRC)	CD45	Yes
P11049	Leukocyte antigen CD37	CD37	Yes
P11279	Lysosome-associated membrane glycoprotein 1 (LAMP1)	CD107a	Yes
Q9BSA4	Protein tweety homolog 2 (TTYH2)	---	Yes

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Supplemental Table 9: Proteins not detected in patient MM samples by PRM.

Uniprot ID	Description	CD protein	Detected by PRM?
O43278	Kunitz-type protease inhibitor 1 (SPINT1)	---	No
O75629	Protein CREG1	---	No
P10747	T-cell-specific surface glycoprotein CD28	CD28	No
P15391	B-lymphocyte antigen CD19	CD19	No
P30203	T-cell differentiation antigen CD6	CD6	No
P32004	Neural cell adhesion molecule L1 (L1CAM)	CD171	No
P33527	Multidrug resistance-associated protein 1 (ABCC1)	---	No
P36888	Receptor-type tyrosine-protein kinase FLT3	CD135	No
P43121	Cell surface glycoprotein MUC18 (MCAM)	---	No
Q13201	Multimerin-1 (MMRN1)	---	No
Q5QGZ9	C-type lectin domain family 12 member A (CLEC12A)	---	No
Q96DU3	SLAM family member 6 (SLAMF6)	---	No
Q9H7F0	Probable cation-transporting ATPase 13A3 (ATP13A3)	---	No
Q9HA82	Ceramide synthase 4 (CERS4)	---	No

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375 **Supplemental Table 10**: Percent expression of MM antigens on CD138+ patient cells by FCM

Antigen	Patient 10	Patient 16	Patient 18	Patient 19
CD5	0.01%	1.92%	5.63%	1.07%
CD98hc	1.0%	5.2%	17.3%	12.7%
CD147	99.9%	99.9%	96.3%	99.8%
CD166	95.3%	97.8%	51.3%	99.0%
CD205	75.2%	94.0%	25.1%	79.8%

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391 **Supplemental Table 11**: Chromatography and MS instrument acquisition settings for analysis of

392 CSC-Technology Samples

Sample Volume	20 µL	**MS^2 Acquisition**	Data dependent, Top 15 precursor, Centroid
Stationary Phase	C_{18}	**MS^2 Fragmentation**	HCD
NanoLC System	Dionex UltiMate 3000 RSLCnano	**MS^2 Detection**	Orbitrap
LC Solvent A	100% H_2O, 0.1% formic acid	**MS^2 Fixed First Mass**	–
LC Solvent B	80% ACN, 20% H_2O, 0.1% formic acid	**MS^2 Resolution**	17,500 @ 200 *m/z*
Gradient Ramp	2.0 - 27.5% B		
Duration	135 minutes	**Isolation Window**	2.0 *m/z*
Flow Rate	300 nL/min		
Mass Spectrometer	Thermo Q Exactive Orbitrap	**MS^2 AGC target**	5e4
Spray Voltage	2.0 kV	**MS^2 Maximum IT**	110 ms
In-Source CID	0.0 eV	**Normalized Collision Energy**	27
MS^1 Scan Range	350-1600 m/z	**Minimum Intensity Req.**	4500
MS^1 Resolution	70,000 @ 200 *m/z*	**Dynamic Exclusion**	30.0 s
MS^1 AGC Target	1e6		
MS^1 Maximum IT	50 ms		

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Identification of MM immunotherapy targets by MS – Supplemental materials

399 **Supplemental Table 12**: Proteome Discoverer 2.2 search parameters

Platform	ProteomeDiscoverer 2.2	**Static Modifications**	Carbamidomethyl (C)
Search Algorithms	SequestHT, MS Amanda 2.0	**Dynamic Modifications**	Oxidation (M), Acetylation (N-terminus) Deamidation (N) for CSC-Samples only
Validation	Percolator Peptide Validator Protein FDR Validator	**Target FDR (Strict) for PSMs:**	0.01
Database	UniProt; Human; created 10/03/2017	**Target FDR (Relaxed) for PSMs:**	0.05
Digest	Trypsin (semi) 2 Missed Cleavages Allowed	**Target FDR (Strict) for Peptides:**	0.01
Precursor Mass Tolerance	10 ppm	**Target FDR (Relaxed) for Peptides:**	0.05
Fragment Mass Tolerance	0.02 Da		

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Supplemental Table 13: Chromatography and MS instrument acquisition settings for PRM analyses

Sample Volume	20 µL	**MS^1 AGC Target**	4e5
Stationary Phase	C_{18}	**MS^1 Maximum IT**	50 ms
NanoLC System	Dionex UltiMate 3000 RSLCnano	**MS^2 Acquisition**	Targeted, Profile
LC Solvent A	100% H_2O, 0.1% formic acid	**MS^2 Fragmentation**	HCD
LC Solvent B	80% ACN, 20% H_2O, 0.1% formic acid	**MS^2 Detection**	Orbitrap
Gradient Ramp	2.0 - 27.5% B		
Duration	135 minutes	**MS^2 Scan Range**	120-1200 *m/z*
Flow Rate	300 nL/min		
Mass Spectrometer	Thermo Orbitrap Fusion Lumos	**MS^2 Resolution**	30,000 @ 200 *m/z*
Spray Voltage	2.1 kV	**Isolation Window**	1.6 *m/z*
In-Source CID	0.0 eV	**MS^2 AGC Target**	1e5
MS^1 Scan Range	300-1700 m/z	**MS^2 Maximum IT**	120 ms
MS^1 Resolution	120,000 @ 200 *m/z*	**Normalized Collision Energy (Default)**	30

Identification of MM immunotherapy targets by MS – Supplemental materials

Supplemental Figure 1: Matrix of non-CD proteins that were chosen for PRM assay development. For each protein, its detection (observed vs. not observed by CSC) among the B and MM cell lines in the present study is indicated in the first six columns. Detection by CSC among human cell lines described in the CSPA(55) is included for comparison. White squares indicate that data are not available for this protein in the CSPA.

Supplemental Figure 2: Relative abundance of proteins detected by PRM analysis of whole cell lysates of MM and B cell lines. (A) Kunitz-type protease inhibitor 1 (SPINT1), (B) Calumenin (CALU), (C) Adenylate cyclase type 3 (ADCY3), (D) CD205 (LY75), (E) Protein CREG1 (CREG1), (F) Prothrombin (F2), (G) Metalloproteinase inhibitor 1 (TIMP1), (H) Immunoglobulin heavy constant gamma 2 (IGHG2), (I) Transferrin receptor protein 1 (TFR1), (J) Serotransferrin (TF), (K) Phosphatidylcholine-sterol acyltransferase (LCAT), (L) Thy-1 membrane glycoprotein (CD90), (M) Sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1), (N) Sodium/potassium-transporting ATPase subunit beta-1 (ATP1B1), (O) Integrin B2 (ITGB2), (P) Intercellular adhesion molecule 1 (ICAM1) (Q) T-cell surface glycoprotein CD5 (R) 4F2 cell-surface antigen heavy chain (CD98hc) (S) CD45 (PTPRC), (T) Leukocyte antigen CD37, (U) Lysosomal-associated membrane protein 1 (LAMP1, CD107a), (V) Coagulation factor V (F5), (W) Intercellular adhesion molecule 2 (ICAM2), (X) CD44, (Y) Sarcoplasmic/ endoplasmic reticulum calcium ATPase 2 (SERCA2), (Z) Plasma membrane calcium-transporting ATPase 1 (PMCA1), (AA) TATA-box-binding protein (TBP), (AB) Voltage-dependent anion-selective channel protein 1 (VDAC1), (AC) Plasma membrane calcium-transporting ATPase 4 (ATP2B4), (AD) Integrin beta-7 (ITGB7) (AE) Intercellular adhesion molecule 3 (ICAM3), (AF) Multidrug resistance-associated protein 1 (ABCC1), (AG)

Basigin (CD147), (AH) Receptor-type tyrosine-protein kinase FLT3 (FLT3), (AI) Cell surface glycoprotein MUC18 (MCAM), (AJ) Translocon-associated protein subunit alpha (SSR1), (AK) Lipopolysaccharide-responsive and beige-like anchor protein (LRBA), (AL) Ephrin-B2 (EFNB2) (AM) Sodium/potassium-transporting ATPase subunit beta-3 (ATP1B3), (AN) Microfibril-associated glycoprotein 4 (MFAP4), (AO) Histone H3.3 (H3F3A), (AP) Transcription factor A, mitochondrial (TFAM), (AQ) Tumor necrosis factor receptor superfamily member 17 (BCMA), (AR) Acid ceramidase (ASAH1), (AS) CD166 (ALCAM), (AT) P-selectin glycoprotein ligand 1 (SELPLG), (AU) C-type lectin domain family 12 member A (CLEC12A), (AV) Osteoclast-associated immunoglobulin-like receptor (OSCAR), (AW) Prenylcysteine oxidase-like (PCYOX1L), (AX) SLAM family member 6 (SLAMF6), (AY) Ceramide synthase 2 (CERS2), (AZ) Cleft lip and palate transmembrane protein 1-like protein (CLPTM1L), (BA) Protein tweety homolog 2 (TTYH2), (BB) Probable cation-transporting ATPase 13A3 (ATP13A3), (BC) Transmembrane protein 206 (TMEM206), (BD) Ceramide synthase 4 (CERS4), (BE) Adenosine deaminase 2 (ADA2), (BF) Calmodulin-like protein 5 (CALML5), (BG) Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), (BH) N-acetylglucosamine-1-phosphotransferase subunit gamma (GNPTG), (BI) Bis(5'-adenosyl)-triphosphatase ENPP4 (ENPP4) were detectable by PRM in whole cell lysates from the B and MM cell lines. Individual B cell line peak areas are shown in green, and individual MM cell line peak areas are shown in blue. Abundance for a pooled sample control, comprising all 6 cell lines, is shown in red at the left of each graph.

Supplemental Figure 3: Relative abundance of proteins detected by PRM analysis of whole cell lysates of primary human MM patient samples. (A) Calumenin (CALU), (B) Adenylate

cyclase type 3 (ADCY3), (C) CD205 (LY75), (D) Prothrombin (F2), (E) Metalloproteinase inhibitor 1 (TIMP1), (F) Immunoglobulin heavy constant gamma 2 (IGHG2), (G) Transferrin receptor protein 1 (TFR1), (H) Serotransferrin (TF), (I) Phosphatidylcholine-sterol acyltransferase (LCAT), (J) Thy-1 membrane glycoprotein (CD90), (K) CD3 delta (CD3D), (L) Sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1), (M) Sodium/potassium-transporting ATPase subunit beta-1 (ATP1B1), (N) Integrin B2 (ITGB2), (O) CD45 (PTPRC), (P) CD37, (Q) Lysosomal-associated membrane protein 1 (LAMP1, CD107a), (R) CD20, (S) Coagulation factor V (F5), (T) Intercellular adhesion molecule 2 (ICAM2), (U) CD44, (V) Sarcoplasmic/ endoplasmic reticulum calcium ATPase 2 (SERCA2), (W) Plasma membrane calcium-transporting ATPase 1 (PMCA1), (X) TATA-box-binding protein (TBP), (Y) Voltage-dependent anion-selective channel protein 1 (VDAC1), (Z) Plasma membrane calcium-transporting ATPase 4 (ATP2B4), (AA) Intercellular adhesion molecule 3 (ICAM3), (AB) Translocon-associated protein subunit alpha (SSR1), (AC) Sodium/potassium-transporting ATPase subunit beta-3 (ATP1B3), (AD) Microfibril-associated glycoprotein 4 (MFAP4), (AE) Histone H3.3 (H3F3A), (AF) Transcription factor A, mitochondrial (TFAM), (AG) Tumor necrosis factor receptor superfamily member 17 (BCMA), (AH) Acid ceramidase (ASAH1), (AI) P-selectin glycoprotein ligand 1 (SELPLG), (AJ) Osteoclast-associated immunoglobulin-like receptor (OSCAR), (AK) Prenylcysteine oxidase-like (PCYOX1L), (AL) Peroxidasin homolog (PXDN), (AM) Ceramide synthase 2 (CERS2), (AN) Sortilin (SORT1), (AO) Protein tweety homolog 2 (TTYH2), (AP) Transmembrane protein 206 (TMEM206), (AQ) Adenosine deaminase 2 (ADA2), (AR) Calmodulin-like protein 5 (CALML5), (AS) Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), (AT) N-acetylglucosamine-1-phosphotransferase subunit gamma (GNPTG), (AU) Bis(5'-adenosyl)-

triphosphatase ENPP4 (ENPP4) were detectable by PRM in the patient samples. Individual patient peak areas are shown in blue, where dark shading represents the CD138+ fraction, and light shading represents the CD138- fraction. The average and standard deviation of the 6 patients per condition is shown at the left in black (CD138+) and white (CD138-). Abundance for a pooled sample control, comprising all 6 cell lines, is shown in red at the right of each graph. This pooled sample was not included in statistical comparisons and is included as a control. The mean total fragment ion peak areas of the six patients' CD138+ and CD138– samples were compared using a parametric ratio paired t-test. Statistical significance is assigned by p-value <0.05. On graphs, p-value represented by annotations: n.s. for $p > 0.05$, * for $p<0.05$, ** for $p<0.01$, *** for $p<0.001$, **** for $p<0.0001$.

Supplemental Figure 4: Flow cytometric analyses of target antigen expression in normal hematopoietic cells

Expression of the 5 candidate antigens in (A) whole normal BM cells, (B) freshly purified peripheral blood CD19+ normal B cells, and (C) freshly purified peripheral blood CD3+ normal T cells from healthy donors. The x-axis shows $\log^{10}$ fluorescence intensities for each antibody, while the y-axis shows cell counts normalized to maximum of cells collected for each sample (20,000 cells per sample). Staining with antibody is shown as open histograms, and isotype staining as shaded histograms.