SUPPLEMENTAL MATERIALS:

Supplemental Figure 1. FACS analysis of Cell surface expression of CD46 on myeloma cell line panel.

Supplemental Figure 2. Extracellular CD46 antigen does not have appreciable shedding from cell surface and no antibody-stimulated shedding was observed.

Supplemental Figure 3. Antibody affinity for recombinant human CD46.

Supplemental Figure 4. Antibody affinity for living multiple myeloma cells.

Supplemental Figure 5. Potent and selective killing of the RPMI8226 myeloma cell line by anti-CD46 immunotoxin (saporin).

Supplemental Figure 6. HPLC analysis with hydrophobic interaction chromatography for antibody-mcvcpab-MMAF conjugates.

Supplemental Figure 7. Inverse correlation of CD46 transcript and cell surface expression with EC_{50} values of CD46-ADC by in vitro cytotoxicity assays.

Supplemental Figure 8. CD46-ADC cytotoxicity is dependent on interaction with cell surface CD46.

Supplemental Figure 9. Increases in *CD46* gene expression are associated with MM development and FISH 1q gain.

Supplemental Figure 10. Copy number correlation analysis for *CD46*, *CKS1B*, *MCL1*, and the control *CCND1*.

Supplemental Figure 11. Evaluation of cell surface CD46 expression in primary MM cells from patients with amp1q21 and normal 1q.

Supplemental Figure 12. Representative examples of FACS analysis of CD46 of various cell populations in myeloma patient bone marrow aspirates.

Supplemental Figure 13. Effect of CD46-ADC on myeloma cells and normal bone marrow mononuclear cells at concentrations up to 100 nM.

Supplemental Figure 14: Histologic analysis of tissues from CD46-ADC-treated transgenic mice expressing human CD46.

Supplemental Table 1. Clinical characteristics of MM patients tested for CD46 expression.

Supplemental Table 2. CD46 antigen density quantitation for bone marrow cell populations from myeloma patients.
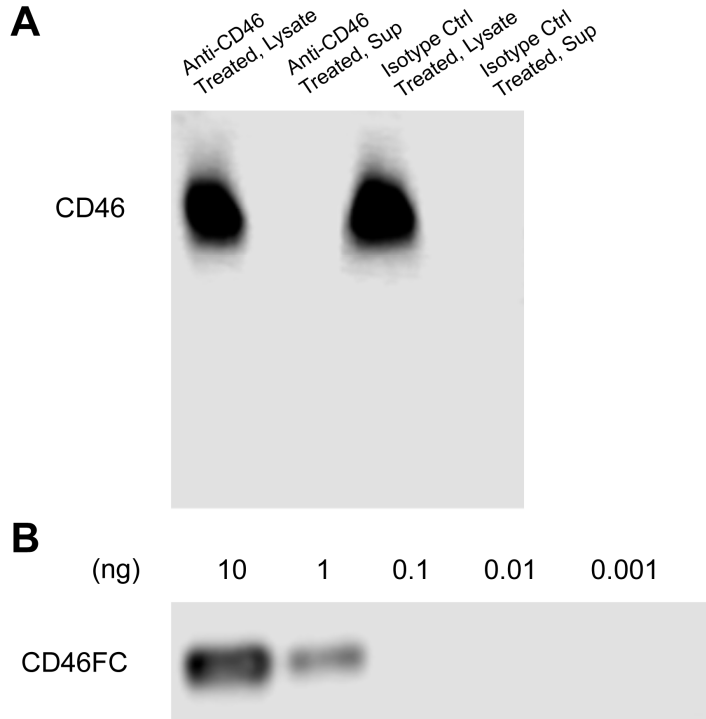
Supplemental Table 3. CD46 antigen density quantitation for bone marrow cell populations from normal donors.

Supplemental Table 4. CD46 antigen density quantitation for peripheral blood cell populations from normal donors.

Supplementary Methods. Data Mining Procedures.
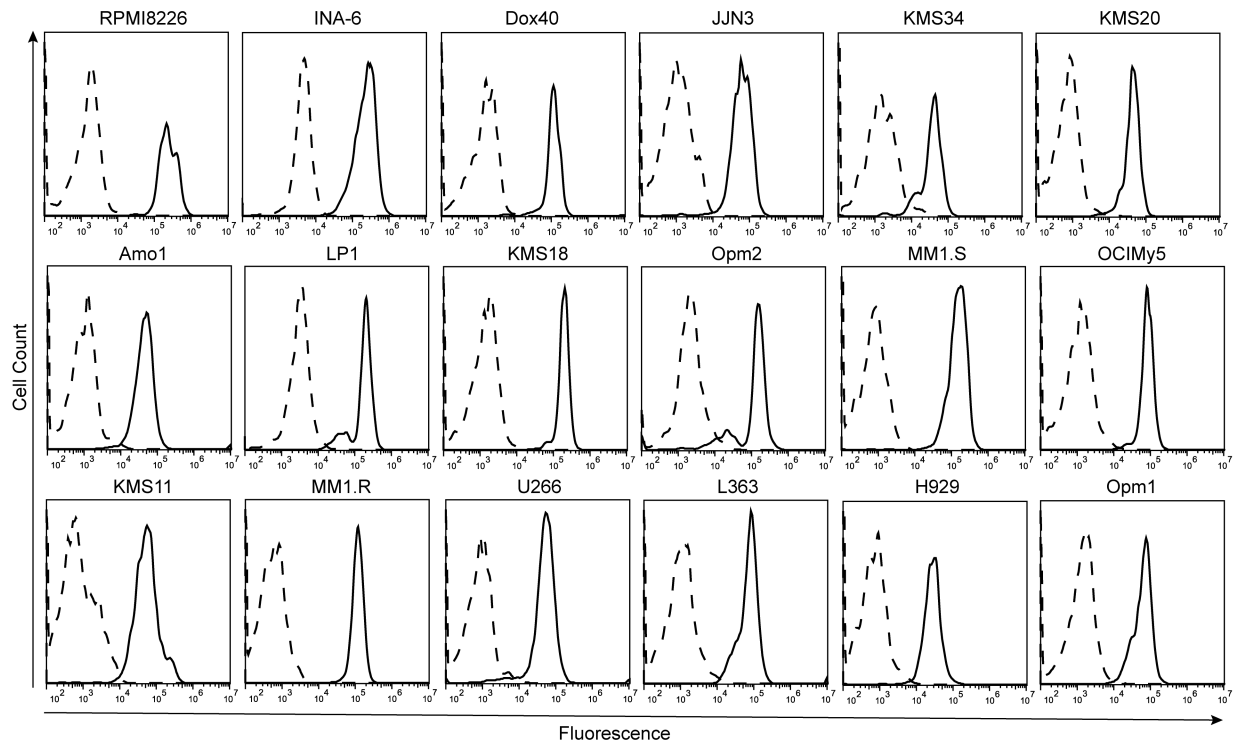
Supplemental Figure 1. FACS analysis of cell surface expression of CD46 on MM cell line panel. CD46 expression on the surface of 18 MM cell lines was measured by conventional FACS with biotinylated anti-CD46 antibody and streptavidin conjugated secondary antibody (solid lines). A nonbinding biotinylated antibody was used as a control (dashed lines). Representative data from 3 experiments.

A

Anti-CD46 Treated, Lysate Anti-CD46 Treated, Sup Isotype Ctrl Treated, Lysate Isotype Ctrl Treated, Sup

CD46

B

| (ng)	10	1	0.1	0.01	0.001

CD46FC

Supplemental Figure 2. Extracellular CD46 antigen does not have appreciable shedding from cell surface and no antibody-stimulated shedding was observed. (A) To assess whether CD46 antigen is actively shed from the cell surface, 4×10^5 RPMI8226 cells were cultured in a 6-well plate for 24 hours, then incubated with anti-CD46 antibody 23AG2 IgG1 or isotype control (Ctrl) antibody YSC10 IgG1 for additional 24 hours. 5ml supernatant (Sup) was concentrated 50x to 100 µl, and 12 µl was loaded per well and western blot performed with commercial anti-CD46 antibody H294 (Santa Cruz Biotechnology). CD46 antigen was readily detectable in cell lysate, but not detected in the supernatant. (B) The detection limit of the shedding experiment above was assessed by loading graded concentrations of the CD46-Fc fusion protein (CD46FC) onto the gel and western blotting with anti-CD46 antibody.

Analysis Data
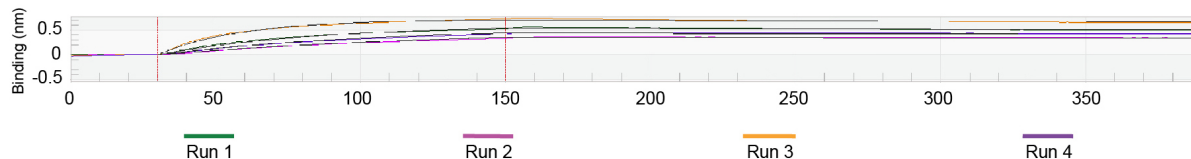
| Index	Sample ID	Conc. (nM)	Information	KD (M)	ka (1/Ms)	ka Error	kd (1/s)	kd Error	Rmax	Rmax Error	R equilibrium
1	HUCD46SCR12FC	200		2.988e-9	8.795e4	8.572e2	2.628e-4	1.46e-5	0.6402	0.005562	0.6308
2	HUCD46SCR12FC	100		2.988e-9	8.795e4	8.572e2	2.628e-4	1.46e-5	0.5858	0.009586	0.5688
3	HUCD46SCR12FC	400		2.988e-9	8.795e4	8.572e2	2.628e-4	1.46e-5	0.7290	0.002503	0.7235
4	HUCD46SCR12FC	150		2.988e-9	8.795e4	8.572e2	2.628e-4	1.46e-5	0.5826	0.006588	0.5712

Supplemental Figure 3. Antibody affinity for recombinant human CD46. (Affinity measurement of anti-CD46 antibody 23AG2 binding to recombinant human CD46 fragment containing Sushi domains 1 and 2 fused to human Fc (designated as HUCD46SCR12FC) using the BLItz system (ForteBio). Biotin-labeled 23AG2 human IgG1 was captured onto streptavidin biosensor (ForteBio). Varying concentrations of recombinant CD46 fragment were used to obtain binding (duration 120 seconds) and dissociation (duration 240 seconds) kinetics. Global fit was used to obtain the KD value as well as on- and off-rates (ka and kd, respectively).
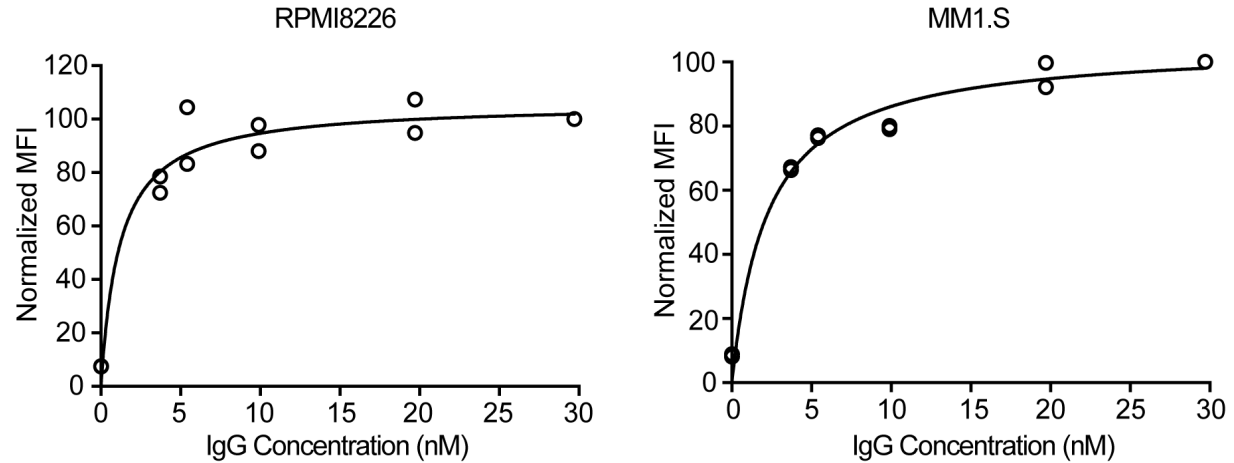
Supplemental Figure 4. Antibody affinity for living multiple myeloma cells. Varying concentrations of the anti-CD46 human IgG1 23AG2 were incubated with MM cell lines RPMI8226 and MM1.S at 4 ^{0}C overnight. Following three washes with PBS/1% BSA, bound antibody was detected by goat anti-human Fc specific antibody conjugated with Alexa Flour® 647 (Jackson ImmunoResearch). MFI values were curve fit using one-site specific binding model (GraphPad) to generate K_D 1.19 nM (SEM 0.44 nM) for RPMI8226; and 2.24 nM (SEM 0.42 nM) for MM1.S. The experiments were performed in duplicate (data points shown as open circles).
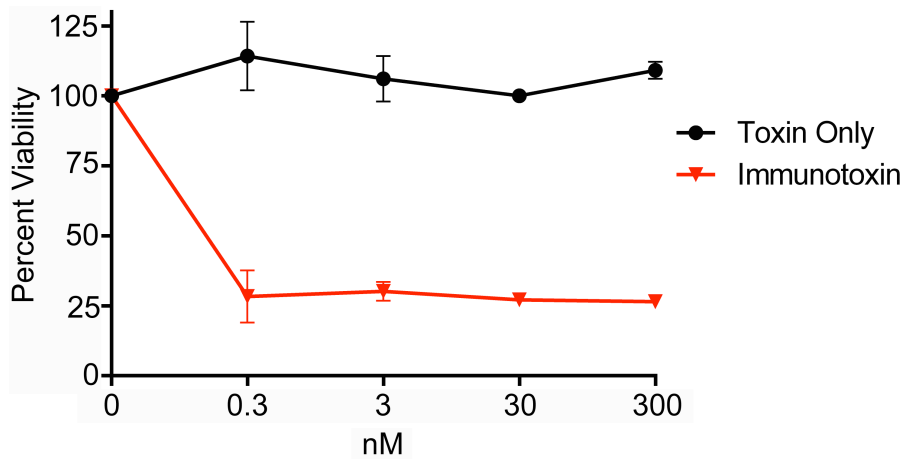
Supplemental Figure 5. Potent and selective killing of the RPMI8226 MM cell line by anti-CD46 immunotoxin. Anti-CD46 human IgG1 23AG2 was biotinylated and mixed at molar ratio 1:1 with streptavidin-ZAP (SA-ZAP) to form an immunotoxin bearing the ribosome inactivating protein saporin (Advanced Targeting System, Inc), and incubated with RPMI8226 cells at 37 $^{\circ}$C for 96 h. As a control, membrane impermeant SA-ZAP alone was also added (toxin only). Potent cytotoxicity was observed with CD46-targeted toxin, with no cytotoxicity observed from the SA-ZAP toxin alone. Data represent mean +/- SEM, n =3.
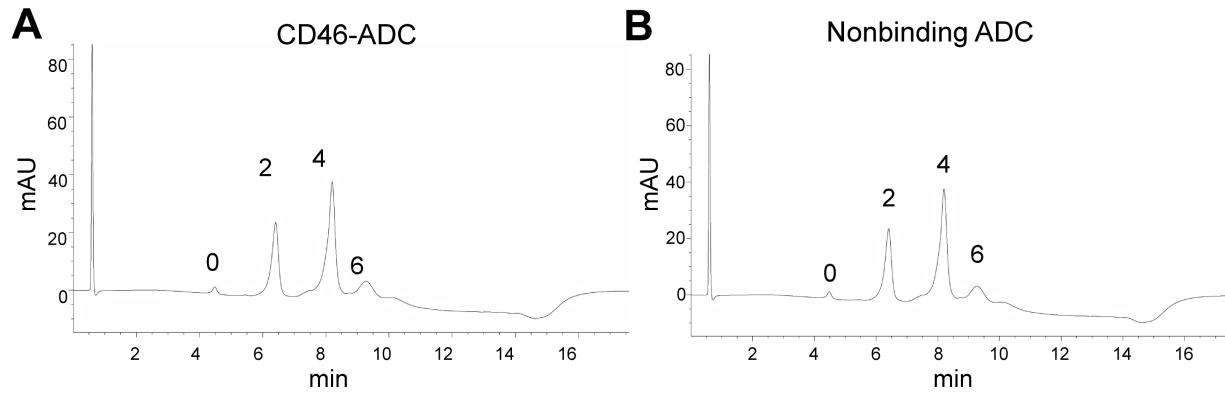
Supplemental Figure 6. HPLC analysis with hydrophobic interaction chromatography for antibody-mcvcpab-MMAF conjugates. (A) Anti-CD46 ADC (23AG2-mcvcpab-MMAF) showed an average drug to antibody ratio of 3.3. Peaks corresponding to 0, 2, 4 and 6 drugs per antibody are shown by numbers on the graphs. (B) For the nonbinding ADC (YSC10-mcvcpab-MMAF), the final conjugate showed an average drug to antibody ratio of 4.1. mAU = milli-absorbance unit.
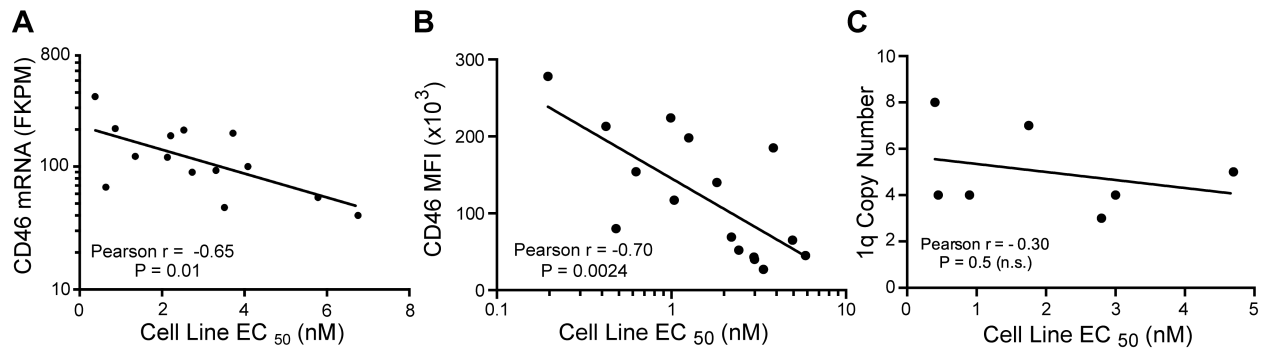
A

CD46 mRNA (FKPM)

Pearson r = -0.65
P = 0.01

Cell Line EC $_{50}$ (nM)

B

CD46 MFI (x10^3)

Pearson r = -0.70
P = 0.0024

Cell Line EC $_{50}$ (nM)

C

1q Copy Number

Pearson r = - 0.30
P = 0.5 (n.s.)

Cell Line EC $_{50}$ (nM)

Supplemental Figure 7. Inverse correlation of CD46 transcript and cell surface expression with EC$_{50}$ values of CD46-ADC by *in vitro* cytotoxicity assays. (A) High CD46 mRNA in myeloma cell lines by mRNA Seq is associated with a lower EC$_{50}$ for CD46-ADC (Pearson r = -0.65, P = 0.01). mRNA seq data from the MMRF MM HMCL69 Cell Line Characterization Project, courtesy Dr. Jonathan Keats (http://www.keatslab.org/data-repository). **(B)** CD46 antigen cell surface expression (MFI by FACS) correlates with CD46-ADC potency (Pearson r = -0.70, p = 0.0024). (C) There is a nonsignificant trend of increased CD46-ADC potency (decreased EC$_{50}$) with 1q21 copy number in myeloma cell lines for which copy number has been reported (2). Comparison of this analysis to our patient sample data is limited by the limited number of cell lines available with known 1q copy number, and by the finding that all but one known myeloma cell line (OCI-MY5) have gain of 1q21.
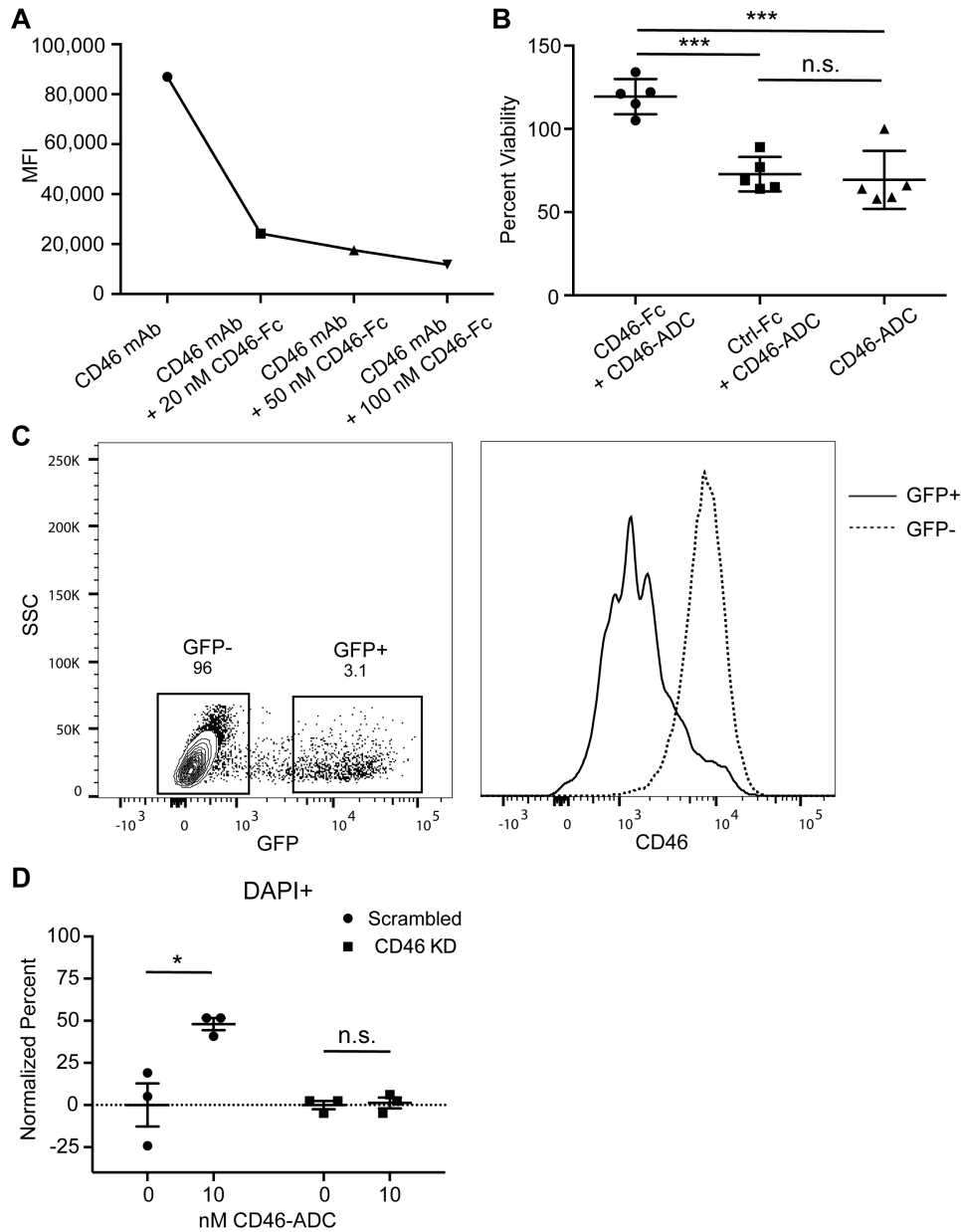
Supplemental Figure 8. CD46-ADC cytotoxicity is dependent on interaction with cell surface CD46. (A) The MFI of CD46 on myeloma cell line RPMI8226 by FACS decreases with the addition of increasing graded concentrations of CD46-Fc fusion containing the CD46-ADC binding epitope. (B) The cytotoxic effect of CD46-ADC (48 hours incubation with RPMI8226) is decreased by addition of CD46-Fc. Student's t test, *** $p < 0.001$, n = 5. (C) Myeloma cell line H929 was infected with GFP and CD46 shRNA coexpressing lentivirus and knockdown of CD46 in GFP+ cells (solid line) was measured by flow cytometry for CD46 compared to the uninfected GFP-negative cells (dotted line). CD46 antigen density was knocked down from 146,647 in uninfected (GFP-negative) cells to 25,847 in GFP+ cells. (D) H929 shRNA transduced cells were incubated for 48 hours with 0 (control) or 10 nM CD46-ADC were assayed for induction of cell death by FACS for DAPI. CD46 Knockdown: cells transduced with CD46 shRNA. Scrambled: cells transduced with scrambled shRNA sequence (control). Student's t test, * $p < 0.05$, n = 3. Data represent mean +/- SEM.

9

A CD46 Expression (Zhan et al. Blood, 2002)

Fold Change: 3.063
P-value: 6.375×10^{-5}

Y-axis: CD46 Transcript Log$_2$ median-centered intensity

X-axis: Normal Plasma Cell, Multiple Myeloma

B CD46 Expression (Zhan et al. Blood, 2007)

p<0.001
p<0.01

Y-axis: CD46 Transcript Log$_2$ median-centered intensity

X-axis: Normal Plasma Cell, MGUS, Smoldering MM

C CD46 Expression (Agnelli et al. GenChrCan, 2009)

Fold Change: 3.896
P-value: 0.006

Y-axis: CD46 Transcript Log$_2$ median-centered intensity

X-axis: Normal Plasma Cell, Multiple Myeloma

D CD46 Copy Number (Agnelli et al. GenChrCan, 2009)

p=0.0003

Y-axis: Log$_2$ CD46 Copy Number

X-axis: No Gain 1q, Gain 1q

E CD46 Expression (Agnelli et al. GenChrCan, 2009)

Fold Change: 2.795
p=0.002

Y-axis: CD46 Transcript Log$_2$ median-centered intensity

X-axis: No Gain 1q, Gain 1q

Supplemental Figure 9. Increases in *CD46* gene expression are associated with MM development and FISH 1q gain. (A-C) Data mined from published gene expression studies show an increase of CD46 mRNA in MM cells (A & C) and through progression from monoclonal gammopathy of unknown significance (MGUS) to smoldering MM compared to normal plasma cells (B) (31-33). Published gene expression data from Agnelli et al. also shows increases in *CD46* copy number (D) and CD46 transcript level (E) in myeloma patients with gain of chromosome 1q21 compared to normal 1q21 status (33). P values were calculated by t-test (A, C-E) or one-way ANOVA analyses with Bonferroni's multiple comparisons test (B).
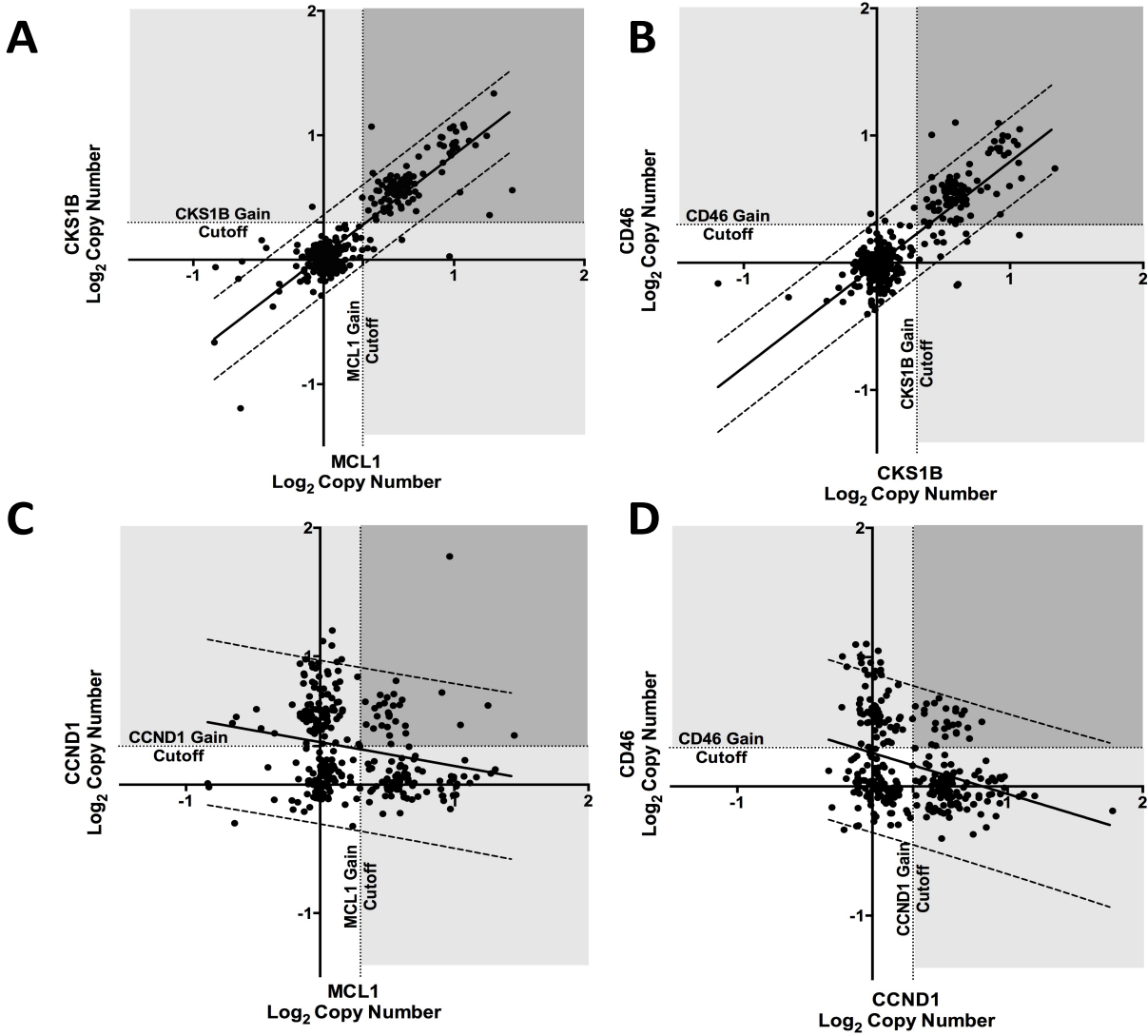
Supplemental Figure 10. Copy number correlation analysis for *CD46*, *CKS1B*, *MCL1*, and the control *CCND1*. Dot-plot correlations of Log$_2$ CGH values in newly diagnosed MM samples for (A) *CKS1B* and *MCL1* (B) *CD46* and *CKS1B* (C) *CCND1* and *MCL1* and (D) *CD46* and *CCND1*. Copy numbers on proximal *MCL1* and *CKS1B* loci are highly correlated and cluster by copy-number quanta. The more distal *CD46* correlates similarly to *MCL1*. The *CCND1* locus, located on a separate chromosome, does not correlate to *MCL1* or *CD46* copy status.
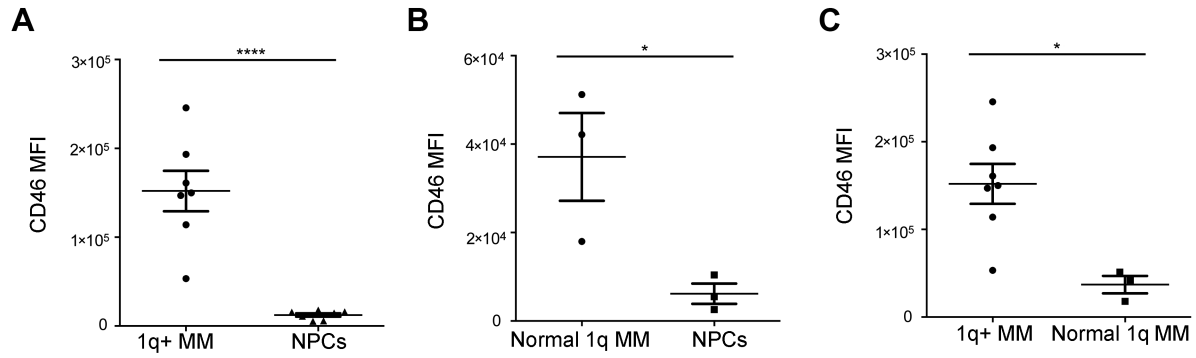
Supplemental Figure 11. Evaluation of cell surface CD46 expression in primary MM cells from patients with amp1q21 and normal 1q. Graph showing CD46 surface expression by MFI from MM cells and matching NPCs. (A) MM (MFI 152,049 ± 22,767) vs. NPC (MFI 12,347 ± 1,914) comparison for 7 patients with amp1q21. (B) MM (MFI 37,113 ± 9,926) vs. NPC (MFI 6,164 ± 2,294) comparison for 3 patients with normal 1q21. (C) CD46 expression is further upregulated in amp1q21 compared to patients with normal 1q. Two-tailed Student's t test, * p < 0.05, *** p < 0.001.
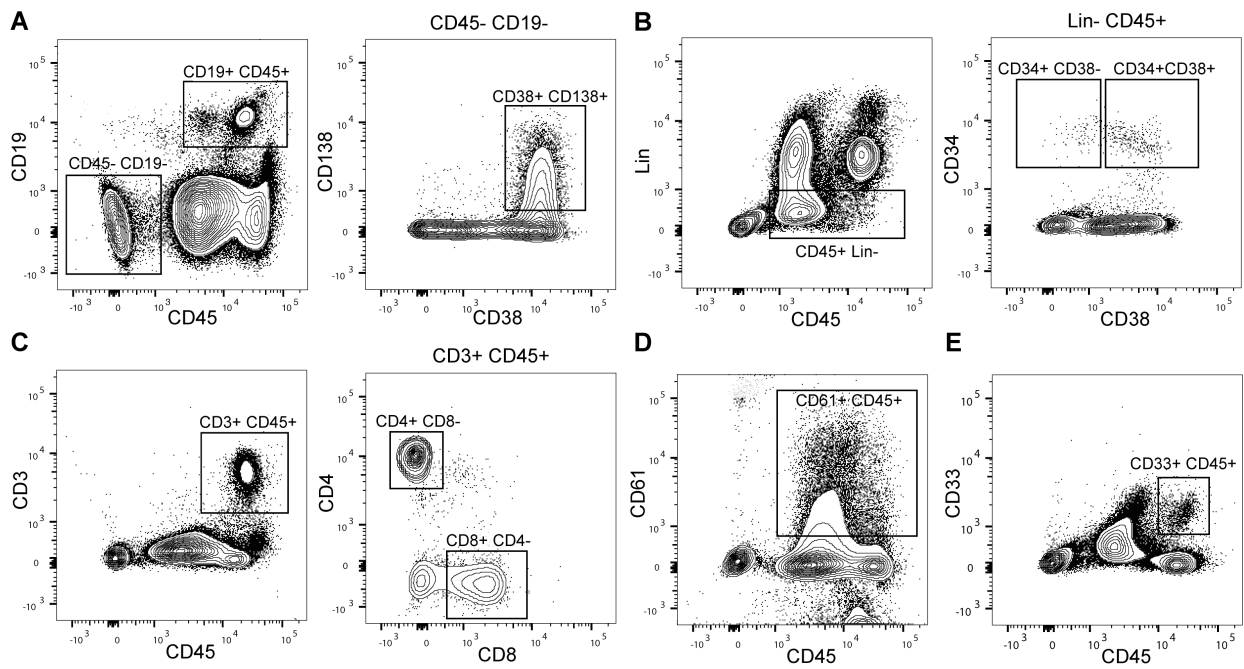
Supplemental Figure 12. Gating strategy for the FACS analysis of CD46 of various cell populations in myeloma patient bone marrow aspirates. (A) MM cells gated as CD45-/CD19-/CD138+/CD38+ and B-cells gated as CD19+/CD45+. (B) HSCs gated as CD34+/CD38-/Lin-/CD45+ and progenitors as CD34+/CD38+/Lin-/CD45+. (C) Cytotoxic T-cells gated as CD8+/CD4-/CD3+/CD45+ and Helper T-cells as CD4+/CD8-/CD3+/CD45+. (D) Megakaryocytes gated as CD61+/CD45+. (E) Granulocytes gated as CD33+/CD45+.
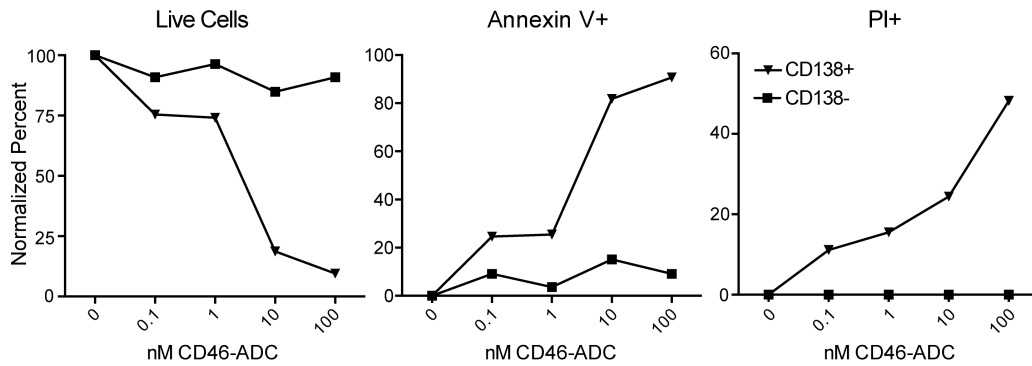
Supplemental Figure 13. Effect of CD46-ADC on myeloma cells and normal bone marrow mononuclear cells at concentrations up to 100 nM. Induction of apoptosis and cell death of CD138+ selected primary MM samples that were gated for high CD38 expression (CD138+, CD38+). The normalized percent change compared to control of Live, Annexin V+ and PI+ populations in graphed across graded concentrations up to 100 nM CD46-ADC. This shows primary MM cell killing at 48 hours with EC_{50} between 1-10 nM. This is a similar EC_{50} range to that observed in cell proliferation assays with 96 hours incubation (Figure 2B). CD46-ADC does not affect apoptosis and cell death in the CD138-negative/CD38-negative bone marrow mononuclear cells populations for concentrations up to 100 nM (CD138-, CD38-). Data from one patient sample, no replicates available.
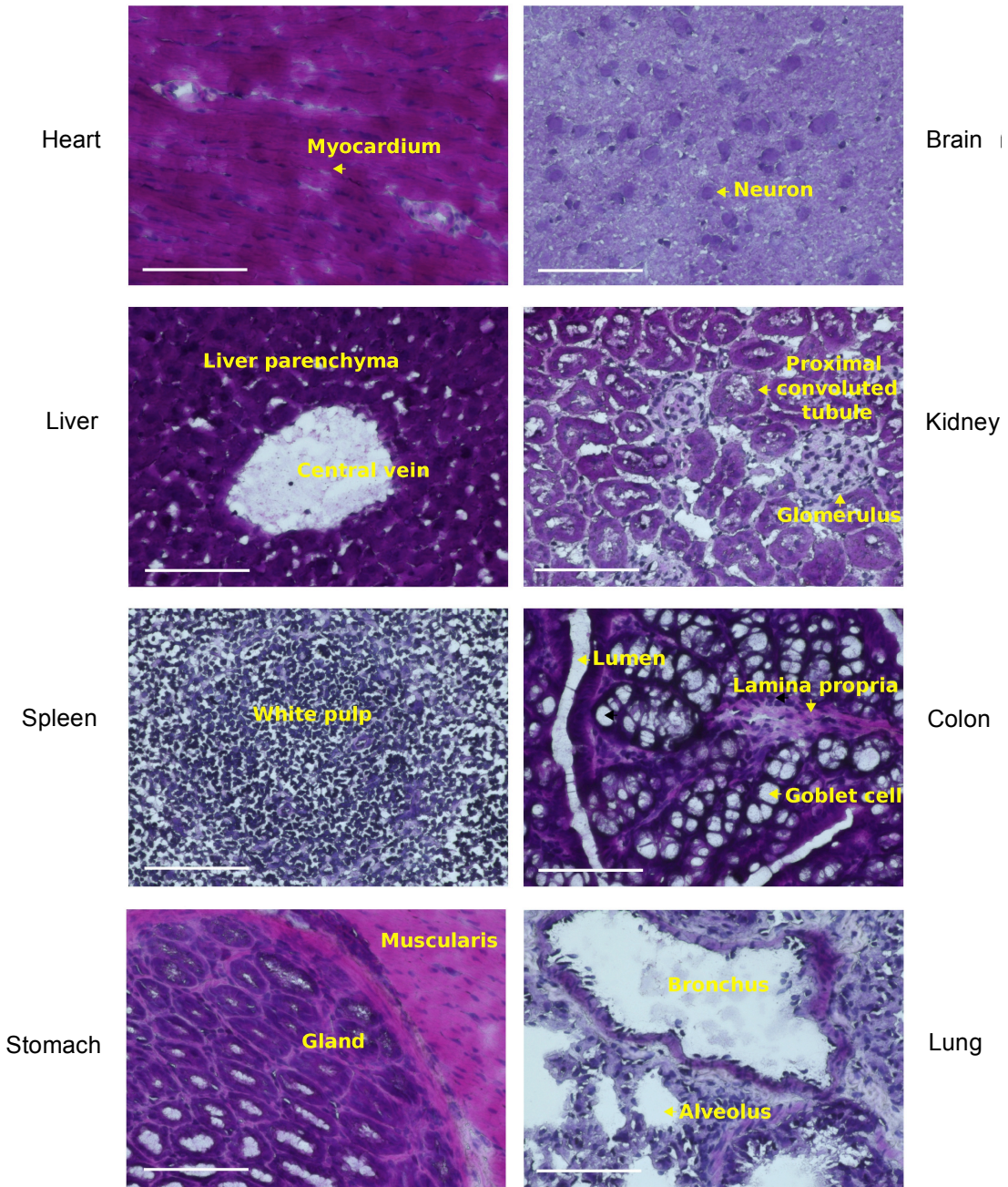
Heart — Myocardium

Brain — Neuron

Liver — Liver parenchyma, Central vein

Kidney — Proximal convoluted tubule, Glomerulus

Spleen — White pulp

Colon — Lumen, Lamina propria, Goblet cell

Stomach — Muscularis, Gland

Lung — Bronchus, Alveolus

Supplemental Figure 14. Histologic analysis of tissues from CD46-ADC-treated transgenic mice expressing human CD46. The experiment is as described in the text and Figure 8 legend. Major organs were formalin-fixed, frozen in liquid nitrogen, cryo-sectioned, and stained by hematoxylin and eosin. Images were taken by a Keyence digital microscope at 40x magnification. Histological features are labeled on the graph. Scale bar, 100 μm.

Supplemental Table 1. Clinical characteristics of MM patients tested for CD46 expression.

Pt #	Age/Sex	Disease State	BM MM cell %	FISH	1q21 copy number	Prior Tx
1	57 m	Relapse	15-20	Normal	2	1
2	73 m	Relapse	50	1q+, Trisomy 13	3-4	2
3	65 m	Relapse	4	Normal	2	6
4	63 f	New Dx	70	del17p, 1q+, 13q-	3	0
5	48 m	New Dx PCL	>90	1q-, 9q+, t(14;16), del17p	1	0
6	57 f	Relapse	50	1q+	4	7
7	51 m	New Dx	70-80	1q+, 13q-	3	0
8	42 f	Relapse	70	1q+, del17p, 13q-	3-6	2
9	68 f	Smoldering	50-60	1q+, t(4;14), 13q-	4+	0
10	63 f	New Dx	70-80	1q+, hyperdiploid, 13q-	3	0
11	56 f	New Dx	40	1q+, 11q+, 17+	3	0
12	65 f	New Dx	70	Normal	2	0
13	57 m	Relapse	20	1q+, 11q+	3	1
14	57 m	Relapse	40-50	1q+, del17p	4	9
15	58 m	New Dx	60-75	t(11;14)	2	0
16	59 f	New Dx	90	Normal	2	0
17	70 m	Relapse	40	t(11;14)	2	4
18	36 f	New Dx	80	1q+, 13q-	4	0
19	69 f	Relapse	40-50	1q+	3	1
20	61 m	Relapse	60	Normal	2	10
21	42 f	New Dx	70-80	t(11;14)	2	0
22	45 f	New Dx	40-50	Hyperdiploid	2	0
23	59 f	Relapse	5-10	13q-, 1q+, del17p, 11q+	4	2
24	63 m	MGUS	0	N/A	N/A	0
25	75 m	Remission	0	t(11;14)	1	2
26	51 m	New Dx	10-25	1q+	4	0
27	55 m	Relapse	10	1q+, 11q+	3	1

Samples (Pt) #1-10 were used for FACS in Figure 6B, samples #11-20 were used for cell surface antigen density determination in Figure 6C and samples #21-27 were used for normal cell population antigen density in Figure 6D and Tables S2, S3 and S4. Dx – diagnosis, Pt – patient, m – male, f – female, Prior Tx – each prior line of treatment consisting of contiguous plan of therapy, separated by progression or toxicity, 1q+ - gain of chromosome 1q21, del17p - deletion of chromosome 17p, t – translocation, MGUS – monoclonal gammopathy of undetermined significance.

Supplemental Table 2. CD46 antigen density quantitation for bone marrow cell populations from myeloma patients.

Patient #	21	22	23	24	25	26	27	Mean (SEM)
1q Status	Nml	Nml	1q+	Nml	Nml	1q+	1q+	
MM cells	92,278	63,564	132,820	N/A	N/A	378,436	155,464	164,512 (55,793)
HSCs	19,554	31,068	18,954	16,027	23,168	31,068	26,511	23,764 (2,264)
Progenitors	47,943	23,981	8,642	19,311	14,674	*N/A	26,593	20,163 (5,768)
B-cells	21,641	10,489	10,435	4,568	6,259	8,245	19,516	11,593 (2,465)
CD8+ T-cells	16,528	27,438	18,450	12,996	17,031	38,795	35,407	23,806 (3,832)
CD4+ T-cells	17,478	21,828	18,114	11,603	2,477	13,862	36,628	17,427 (3,960)
Granulocytes	N/A	N/A	50,659	37,078	47,943	14,925	45,636	39,248 (2,254)
Monocytes	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
MKs	29,461	16	9,018	11,369	N/A	36,055	36,312	20,372 (6,342)

N/A: data not available, either not tested for normal populations or not detectable for MM cells in samples from patients in remission. *Negative MFI values after background subtraction were not convertible to an antigen density number, so is also listed here as N/A. HSCs: hematopoietic stem cells. MKs: megakaryocytes. 1q+: gain of chromosome 1q by FISH.

Supplemental Table 3. CD46 antigen density quantitation for bone marrow cell populations from normal donors.

Cells	Markers	Nml 1	Nml 2	Nml 3	Mean	SEM
Plasma Cells	CD38+ CD138+	16,565	32,132	18,728	22,475	4,869
HSCs	CD34+ CD38- Lin- CD45+	15,920	16,465	22,559	18,315	2,128
Progenitors	CD34+ CD38+ Lin- CD45+	13,609	15,765	16,999	15,458	991
B-Cells	CD19+ CD45+	9,057	9,491	6,782	8,443	840
CD8+T-cell	CD3+ CD8+ CD4- CD45+	10,794	11,394	10,979	11,056	177
CD4+ T-cell	CD3+ CD4+ CD8- CD45+	8,023	13,103	9,382	10,169	1,518
Granulocytes	CD33+ CD45+	41,438	46,246	75,634	54,439	10,688
Monocytes	CD14+ CD45+	44,579	64,823	65,558	58,320	6,874
MKs	CD61+ CD45+	23,339	24,300	23,677	23,772	281

HSCs – hematopoietic stem cells. MKs – megakaryocytes.

Supplemental Table 4. CD46 antigen density quantitation for peripheral blood cell populations from normal donors.

Cells	Markers	Nml 4	Nml 5	Nml 6	Mean	SEM
B-Cells	CD19+ CD45+	6,056	7,106	9,327	7,496	964
CD8+ T-cell	CD3+ CD8+ CD4- CD45+	2,087	3,474	5,532	3,698	1,001
CD4+ T-cell	CD3+ CD4+ CD8- CD45+	7,209	7,927	9,631	8,256	718
Granulocytes	CD33+ CD45+	39,102	33,162	49,305	40,523	4,714
Monocytes	CD14+ CD45+	51,399	38,920	78,393	56,237	11,649
Platelets	CD61+ CD45-	4,940	8,002	1,148	4,697	1,982

Supplemental Methods: Data Mining Procedures

CD46 mRNA expression and copy number data was mined from three published datasets (31-33). mRNA expression data was downloaded as Log_2 median intensities and Log_2 copy number from public Oncomine microarray datasets (https://www.oncomine.org) and analyzed for fold increase using GraphPad Prism v6.0c. Mean intensities and copy number for cohorts in each dataset were analyzed for significance by two-tailed Student's t test or by one-way ANOVA analyses with Bonferroni's multiple comparisons test.

Data from the CoMMpass Study[SM] (Interim Analysis 6) were kindly provided by the Multiple Myeloma Research Foundation (https://research.themmrf.org). Normalized copy number data for 322 patients' tumor DNA was annotated as being amplified where the log_2 ratio of tumor/normal segmented copy number exceeded 0.3. Copy number values were summarized for Figure 5A by dividing chromosome one into 1,000 equally-sized windows and reporting the maximum segmented copy number call within that window. Pearson's correlation was reported. Fractional frequency of co-amplification with MCL1-containing window was calculated for windows along chromosome 1 for each sample.

Correlations between log_2 copy number values for *CD46*, *MCL1*, *CKS1B*, and *CCND1* were conducted using GraphPad Prism v6.0c, with log_2 values of >0.3 denoting copy gain for all loci analyzed. Linear regressions and 95% confidence intervals were also plotted. CD46 expression was analyzed for a subset of 260 patient samples for which both RNA-seq and Array-CGH data were available. CD46 expression is quantified as FPKM. Mean CD46 FPKM values ± 95%CI were plotted and analyzed for the whole population (260 patients), as well as for cohorts defined as *CD46* or *MCL1* copy number gain (log_2 copy number greater than 0.3 for each loci) or no gain (log_2 copy number less than 0.3 for each loci). CD46 FPKM values for each cohort were compared for significance by one-way ANOVA, using Tukey's multiple comparison correction using GraphPad Prism v6.0c.