Supplemental Information

Comprehensive CRISPR-Cas9 screens identify genetic determinants of drug responsiveness in multiple myeloma

Stephan R. Bohl[1,4*], Laura K. Schmalbrock[1,2,6*], Imke Bauhuf[1*], Tatjana Meyer[1], Anna Dolnik[2], Martin Szyska[2], Tamara J. Blätte[2], Sarah Knödler[1], Linda Röhner[1], Denise Miller[1], Miriam Kull[1], Christian Langer[3], Hartmut Döhner[1], Anthony Letai[4], Frederik Damm[2], Dirk Heckl[5], Lars Bullinger[2,6], Jan Krönke[1,2,#]

Supplemental Material and Methods

Tumor cell isolation

Plasma cells from bone marrow aspirates were isolated through Ficoll-Hypaque gradient separation. For samples with a plasma cell count <80% and sufficient bone marrow cells enrichment with anti-human CD138 antibody coated magnetic beads (Miltenyi Biotec) was performed.

Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) in combination with immunofluorescent detection of light-chain restricted plasma cells (cIg-FISH) was performed in plasma cells according to standardized protocols using commercially available probes (Abbott Molecular; MetaSystems).

Region	FISH probe	Company
1p32/1q21	MetaSystems XL 1p32/1q21	Metasystems
13q14	Vysis LSI 13 (13q14) SpectrumGreen Probe	Abbott
17p13.1	Vysis LSI TP53 (17p13.1) Spectrum Orange Probe	Abbott
9q34	Vysis 9q34 Spectrum Aqua FISH Probe	Abbott
14q32/14q32	Vysis LSI IGH Dual Color, Break Apart	Abbott
t(4;14)	Vysis IGH/FGFR3 DF FISH Probe Kit	Abbott
t(14;16)	Vysis IGH/MAF DF FISH Probe Kit	Abbott
t(11;14)	Vysis IGH/CCND1 XT	Abbott
t(8;14)	Vysis LSI IGH/MYC/CEP8 Dual Fusion Probe	Abbott

Information about chromosomal aberrations using standard cIgFISH analyses was available for all patients at diagnosis (n=16) and in 15 of 21 follow-up samples. An overview about the availability of cytogenetic analysis and the presence of cytogenetic alterations at diagnosis and relapse is given in supplemental figure 1.

Validation of selected mutations with ultra-deep targeted re-sequencing

To determine whether mutations found at relapse pre-existed in small subclones in the pre-treatment samples, we performed ultra-deep targeted re-sequencing for selected genes (*ANKMY2, DDB1, RAD54b, TP53*). Short fragments of 100–200 base pairs including the candidate single-nucleotide variants were PCR-amplified (primer are listed in supplemental table S12) from gDNA and subsequently pooled for library construction. Libraries were indexed using the NEBNext Ultra DNA Library Prep Kit (New England Biolabs) and paired-end sequencing on a MiSeq using the MiSeq Reagent Kit v2 (300 cycle, Illumina) was performed as previously described.[2] The mean on-target read depth for all samples was 5182 reads. Variant calling and calculation of the VAF was performed with an in-house analysis pipeline. Mutations were considered true with a VAF >1% or if supported by >10 reads. Mutations with VAFs below 0.1% were manually checked in Integrated Genome Browser (http://software.broadinstitute.org/software/igv/).

Determination of the stability of mutations

We defined mutations that were only present at diagnosis or decreased >10% VAF as ‘loss’ and mutations that were only present at disease progression or increased >10% VAF as ‘gain’, other mutations were defined as ‘stable’. We calculated the stability of mutations by dividing the number of stable mutations by the total number of mutations in both samples.

Clonal evolution fishplots

To visualize the clonal evolution of mutations in individual patients, we generated fishplots using the fishplot package for R[1]. Mutations were organized in mutation clusters according to the VAF changes at diagnosis/progress. Mutation cluster were considered to correspond to a clone with similar VAF changes between diagnosis, and progress (e.g. mutations present at diagnosis/progress, loss or gain of mutations at

progress). For each cluster, we estimated a representative VAF by the median of the cluster mutation VAFs. Clones were considered paternal if the median VAF was higher compared to the representative VAF of subclones or the sum of subclonal VAFs at each time point.

Cell culture and viability assay

MM1S, KMS-27, NCI-H929, OPM2 and RPMI8226 cells (obtained at DSMZ) and maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and cultured in a humidified (37 °C, 5% CO2) incubator. For stable Cas9 expression, cells were transduced with the lentiviral vectors pLKO5d.SSF.SpCas9.P2a.BSD or pLKO5d.EFS.SpCas9.P2A.PAC and selected with blasticidin or puromycin, respectively. The expression of the Cas9 was confirmed by western blot and the activity of the Cas9 was validated by sgRNAs targeting the two pan-essential genes *RPL15* and *SF3B3*. For viability assays, cells were treated for four and seven days with the respective drugs and cell viability was measured by CellTiter-Glo® Luminescent Cell Viability Assay (Promega, USA).

sgRNA library cloning

The following plasmids were used as previously described: pLKO5.hU6.sgRNA.dTom and pLKO5.sgRNA.EFS.PAC[4]. SgRNAs were designed using the GPP sgRNA Designer of the Broad Institute (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design). The sgRNA sequences of the library were cloned into the BsmBI site of the pLKO5.hU6.sgRNA.dTom vector[5]. SgRNA sequences and primers are listed in supplemental table 2 and 4. For single sgRNA validation, each selected sgRNA based on the screening results was individually cloned into pLKO5.sgRNA.EFS.PAC for further analysis.

Lentivirus production

HEK293T cells were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin and transfected with the lentiviral sgRNA library together with the second-generation packaging plasmids psPAX2 (Addgene plasmid 12260) and pMD2.G (Addgene plasmid 12259). After 48 hours, lentiviral containing media was harvested, filtered (0.45 µM pore, PVDF), and immediately used to infect the specified cell line in the presence of 2µg/ml polybrene (Polybrene, Life Technologies).

CRISPR-Cas9 knockout screen

In our CRISPR-Cas9 screens the mean read counts per million reads for each cell line at baseline were highly consistent in all five cell lines. The different concentrations for lenalidomide, bortezomib, dexamethasone, melphalan were based on the IC50 for each cell line (supplemental table S3). In comparison to the positive and negative control sgRNAs [outliers = 0; ROUT (Q=1%)], gene-targeting sgRNAs at the end of each screen provided significant outliers [outliers median = 22 across all cell lines and drugs, ROUT (Q=1%)] determined by the MAGecK software algorithm.

Western blot

Western blot analyses were performed as previously described.[6] The following antibodies were used: Cas9, FANCA, p53, NR3C1, Vinculin, Tubulin, secondary anti-rabbit HRP: 7074S (all from Cell Signaling Technology, Inc).

Annexin V assay

To assess the percentage of cell death in MM1S- and NCI-H929, 1.5×10^6 cells were seeded and treated with different concentrations of melphalan and DMSO as a vehicle control for 4 days. Then, cell death was measured using the Annexin V Apoptosis Detection Kit with Hoechst as living dye (640922, BioLegend, San Diego, CA, USA) according to the manufacturer's protocol.

Statistical analysis

For statistical analysis the unpaired t-test or Mann-Whitney test and for sgRNA ranking the Fisher`s exact test was applied. For all statistical and graphical analysis Graph Pad Prism 7.0 was used (GraphPad Software, San Diego Ca, USA).

Supplemental References:

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2. Arends CM, Galan-Sousa J, Hoyer K, et al. Hematopoietic lineage distribution and evolutionary dynamics of clonal hematopoiesis. 2018. *Leukemia* 32**:** 1908–1919.

3. Miller CA, McMichael J, Dang HX, et al. Visualizing tumor evolution with the fishplot package for R. *BMC Genomics*. 2016;17(1):880.

4. Heckl D, Kowalczyk MS, Yudovich D, et al. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. *Nat Biotechnol*. 2014;32(9):941-946.

5. Labuhn M, Perkins K, Matzk S, et al. Mechanisms of Progression of Myeloid Preleukemia to Transformed Myeloid Leukemia in Children with Down Syndrome. *Cancer Cell*. 2019;36(3):340.

6. Kronke J, Fink EC, Hollenbach PW, et al. Lenalidomide induces ubiquitination and degradation of CK1alpha in del(5q) MDS. *Nature*. 2015;523(7559):183-188.

Supplemental Tables

Supplemental Table S1

Mean coverage of samples analyzed by whole exome sequencing

UPN	Timepoint	Average coverage	Min 1x coverage	Min 10x coverage	Min 50x coverage	Min 100x coverage
1	Diagnosis	115,14	99,65%	96,03%	74,18%	45,25%
1	PB control	59,95	95,41%	80,50%	45,17%	18,98%
1	Relapse	108,97	99,60%	95,53%	71,99%	42,68%
2	Diagnosis	69,97	95,68%	80,61%	48,00%	23,62%
2	PB control	117,71	99,69%	96,34%	75,88%	47,79%
2	Relapse	79,42	95,83%	81,86%	52,58%	28,38%
3	Diagnosis	122,63	99,72%	96,87%	76,97%	49,00%
3	PB control	128,61	99,71%	96,85%	79,15%	53,25%
3	Relapse_1	125,18	99,72%	96,99%	78,60%	51,14%
3	Relapse_2	55,80	93,78%	76,57%	41,39%	17,47%
4	Diagnosis	105,32	99,65%	95,59%	71,78%	42,02%
4	PB control	72,97	99,44%	93,63%	58,69%	23,72%
4	Relapse	119,22	99,69%	96,56%	75,99%	47,73%
5	Diagnosis	80,19	99,51%	94,36%	62,37%	28,21%
5	PB control	54,83	93,68%	79,49%	42,25%	16,15%
5	Relapse	49,60	92,85%	77,64%	37,63%	13,39%
6	Diagnosis	96,66	97,41%	87,65%	61,88%	36,26%
6	PB control	126,96	99,73%	96,82%	78,47%	51,85%
6	Relapse_1	120,23	99,69%	96,35%	76,57%	49,17%
6	Relapse_2	48,84	95,67%	80,65%	37,82%	12,11%
7	Diagnosis	127,14	99,15%	94,89%	76,24%	52,06%
7	PB control	133,33	99,20%	95,31%	78,14%	55,10%
7	Relapse_1	156,27	99,30%	96,24%	82,30%	62,43%
7	Relapse_2	143,68	99,31%	96,22%	81,24%	59,12%
7	Relapse_3	131,34	99,79%	97,88%	82,43%	56,65%
8	Diagnosis	160,00	99,34%	96,24%	82,11%	62,08%
8	PB control	123,92	99,14%	94,80%	75,89%	51,32%
8	Relapse_1	133,36	98,96%	94,95%	78,00%	54,41%
8	Relapse_2	127,84	98,95%	94,90%	77,58%	53,08%
9	Diagnosis	139,86	99,40%	96,07%	80,57%	58,71%
9	PB control	140,20	99,41%	96,20%	81,01%	59,27%
9	Relapse	139,59	99,36%	96,08%	80,70%	58,72%
10	Diagnosis	137,25	99,37%	96,03%	80,13%	57,57%
10	PB control	140,79	99,39%	96,11%	80,67%	58,97%
10	Relapse	142,35	99,38%	95,97%	80,22%	58,04%
11	Diagnosis	142,39	98,99%	95,08%	79,15%	57,34%
11	PB control	140,11	98,95%	95,06%	79,19%	57,13%
11	Relapse_1	107,82	98,66%	93,20%	70,76%	43,66%
11	Relapse_2	148,84	98,99%	95,20%	79,96%	59,23%
13	Diagnosis	137,41	99,33%	96,06%	80,56%	57,86%
13	PB control	142,36	99,36%	96,15%	81,18%	59,72%
13	Relapse	146,73	99,38%	96,32%	81,89%	60,64%
14	Diagnosis	134,49	99,56%	98,05%	84,99%	59,60%
14	PB control	125,52	99,52%	97,45%	81,85%	55,60%
14	Relapse	121,86	99,51%	97,34%	80,81%	53,58%
15	Diagnosis	148,28	99,81%	98,10%	85,30%	63,57%
15	PB control	133,64	99,76%	97,58%	82,32%	58,21%
15	Relapse	140,30	99,80%	97,99%	84,23%	61,01%
16	Diagnosis	154,66	99,56%	98,10%	87,03%	67,34%
16	PB control	122,03	99,44%	96,87%	80,48%	54,88%
16	Relapse	167,56	99,58%	98,41%	89,13%	71,32%
17	Diagnosis	182,99	99,83%	98,46%	88,86%	72,84%
17	PB control	134,61	99,72%	97,22%	82,00%	59,07%
17	Relapse	158,21	99,78%	97,86%	85,36%	65,79%

UPN=unique patient number; Min=minimum

Supplemental Table S2
List of sgRNAs used in the CRISPR-Cas9 screens
Excel file: Bohl et al supplemental table S2

Supplemental Table S3
Overview of drug treatment per cell line (day of harvest)

Cell line	Lenalidomide	Bortezomib	Dexamethasone	Melphalan
MM1S	1µM (24)	1nM (24)	60nM (24)	2µM (24
NCI-H929	1µM (35)	1nM (35)	60nM (35)	2µM (35)
KMS27	1µM (24)	1nM (24)	100nM (24)	2µM (24)
OPM2	100nM (24)	2nM (24)	/	0.5µM (24)
RPMI8226	10µM (15)	3nM (15)	1µM (15)	0.5µM (24)

Supplemental Table S4
Primer sequences and barcodes used for PCR amplification for NGS analyses of the CRISPR-Cas9 screens

Primer	Sequence
Fwd primer	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-XXXXXX(barcode)- TTGTGGAAAGGACGAAACACC
Rev primer	CAAGCAGAAGACGGCATACGAGATGATGTGCGCTCTGCCCACT
Barcode 1	ATCACG
Barcode 2	CGATGT
Barcode 3	TTAGGC
Barcode 4	TGACCA
Barcode 5	ACAGTG
Barcode 6	GCCAAT
Barcode 7	CAGATC
Barcode 8	ACTTGA
Barcode 9	GATCAG
Barcode 10	TAGCTT
Barcode 11	GGCTAC
Barcode 12	CTTGTA
Barcode 13	AGTCAA
Barcode 14	AGTTCC
Barcode 15	ATGTCA
Barcode 16	CCGTCC
Barcode 17	GTAGAG
Barcode 18	GTCCGC
Barcode 19	GTGAAA
Barcode 20	GTGGCC

Supplemental Table S5

Multiple Myeloma patient characteristics
Excel file: Bohl et al supplemental table S5

Supplemental Table S6A

Variant allele frequencies (VAF) of mutations found recurrently acquired at disease progression. Given are the VAFs at diagnosis and all available follow-up relapse samples.

UPN	Time	Gene	DNA change	Protein change	VAF
3	D	DNAH5	c.C4460T	p.A1487V	41,43%
3	R1	DNAH5	c.C4460T	p.A1487V	30,36%
3	R2	DNAH5	c.C4460T	p.A1487V	ND
9	D	DNAH5	c.G5833T	p.E1945X	ND
9	R	DNAH5	c.G5833T	p.E1945X	5,60%
10	D	DNAH5	c.A12090T	p.L4030F	ND
10	R	DNAH5	c.A12090T	p.L4030F	39,16%
14	D	DNAH5	c.G13328A	p.W4443X	ND
14	R	DNAH5	c.G13328A	p.W4443X	7,62%
2	D	WSCD2	c.C161T	p.A54V	ND
2	R	WSCD2	c.C161T	p.A54V	8,71%
3	D	WSCD2	c.C491A	p.A164D	ND
3	R1	WSCD2	c.C491A	p.A164D	ND
3	R2	WSCD2	c.C491A	p.A164D	16%
10	D	WSCD2	c.C742A	p.P248T	0,84%
10	R	WSCD2	c.C742A	p.P248T	45,87%
7	D	TP53	c.G473A	p.R158H	ND
7	R1	TP53	c.G473A	p.R158H	37,39%
7	R2	TP53	c.G473A	p.R158H	45,88%
7	R3	TP53	c.G473A	p.R158H	66,58%
8	D	TP53	c.T581G	p.L194R	ND
8	R1	TP53	c.T581G	p.L194R	0,34%
8	R2	TP53	c.T581G	p.L194R	27,85%
8	D	TP53	c.G743A	p.R248Q	ND
8	R1	TP53	c.G743A	p.R248Q	ND
8	R2	TP53	c.G743A	p.R248Q	29,85%
8	D	TP53	c.G711A	p.M237I	ND
8	R1	TP53	c.G711A	p.M237I	ND
8	R2	TP53	c.G711A	p.M237I	17,86%
9	D	TP53	c.G524A	p.R175H	ND
9	R	TP53	c.G524A	p.R175H	19,32%
14	D	TP53	c.G596A	p.G199E	38,87%
14	R	TP53	c.G596A	p.G199E	14,64%
14	D	TP53	c.A488G	p.Y163C	ND
14	R	TP53	c.A488G	p.Y163C	12,23%
14	D	TP53	c.C817T	p.R273C	ND
14	R	TP53	c.C817T	p.R273C	7,66%
2	D	KRAS	c.A183C	p.Q61H	ND
2	R	KRAS	c.A183C	p.Q61H	19,23%
4	D	KRAS	c.G436A	p.A146T	8,36%
4	R	KRAS	c.G436A	p.A146T	30,61%
8	D	KRAS	c.G35A	p.G12D	43,29%
8	R1	KRAS	c.G35A	p.G12D	30,50%
8	R2	KRAS	c.G35A	p.G12D	52,15%
9	D	KRAS	c.G35A	p.G12D	33,18%
9	R	KRAS	c.G35A	p.G12D	ND
9	D	KRAS	c.A183C	p.Q61H	10,15%
9	R	KRAS	c.A183C	p.Q61H	6,61%
9	D	KRAS	c.G38A	p.G13D	3,99%
9	R	KRAS	c.G38A	p.G13D	ND
14	D	KRAS	c.A183T	p.Q61H	36,49%
14	R	KRAS	c.A183T	p.Q61H	25,33%

Supplemental Table S6B

Presence of selected mutations at diagnosis (D) and relapse (R) determined with ultra-deep targeted sequencing (TDS)

UPN	Time	Gene	Exon	DNA change	Protein change	VAF WES*	VAF TDS*
1	D	ANKMY2	exon3	c.G247A	p.A83T	ND	ND
1	R	ANKMY2	exon3	c.G247A	p.A83T	40,17%	47,40%
3	D	DDB1	exon6	c.685dupG	p.A229fs	ND	1,14%
3	R1	DDB1	exon6	c.685dupG	p.A229fs	0,89%	0,86%
3	R2	DDB1	exon6	c.685dupG	p.A229fs	30,44%	NA
7	D	RAD54B	exon4	c.C322G	p.L108V	ND	ND
7	R1	RAD54B	exon4	c.C322G	p.L108V	25,25%	28,29%
7	R2	RAD54B	exon4	c.C322G	p.L108V	21,95%	27,91%
7	R3	RAD54B	exon4	c.C322G	p.L108V	22,23%	36,99%
8	D	TP53	exon6	c.T581G	p.L194R	ND	0,16%
8	R1	TP53	exon6	c.T581G	p.L194R	0,34%	0,06%
8	R2	TP53	exon6	c.T581G	p.L194R	27,85%	35,46%
8	D	TP53	exon7	c.G743A	p.R248Q	ND	NA
8	R1	TP53	exon7	c.G743A	p.R248Q	ND	0,16%
8	R2	TP53	exon7	c.G743A	p.R248Q	29,85%	30,76%
8	D	TP53	exon7	c.G711A	p.M237I	ND	NA
8	R1	TP53	exon7	c.G711A	p.M237I	ND	ND
8	R2	TP53	exon7	c.G711A	p.M237I	17,86%	NA
9	D	TP53	exon5	c.G524A	p.R175H	ND	0,11%
9	R	TP53	exon5	c.G524A	p.R175H	19,32%	27,09%
14	D	TP53	exon6	c.G596A	p.G199E	38,87%	41,93%
14	R	TP53	exon6	c.G596A	p.G199E	14,64%	NA
14	D	TP53	exon5	c.A488G	p.Y163C	ND	6,70%
14	R	TP53	exon5	c.A488G	p.Y163C	12,23%	21,02%
14	D	TP53	exon8	c.C817T	p.R273C	ND	ND
14	R	TP53	exon8	c.C817T	p.R273C	7,66%	NA

NA=Information not available; ND=Not detected; WES=Whole Exome Sequencing;
TDS=Targeted Deep Sequencing; * Plasma cell corrected variant allele frequency (VAF)

Supplemental Table S7

Genetic alterations in multiple myeloma cell lines (https://depmap.org/portal/)

Cell line	Cytogenetic	NRAS	KRAS	TRAF3	TP53	FAM46C
MM1S	t(14:16) + t(8;14)	WT	MUT	MUT	WT	MUT
NCI-H929	t(4;14)	MUT	WT	WT	WT	MUT
KMS27	t(11;14)	MUT	WT	WT	MUT	WT
OPM2	t(4:14)	WT	WT	WT	MUT	WT
RPMI8226	t(16;22) + t(8;22)	WT	WT	WT	MUT	WT

Supplemental Table S8
List of somatic gene mutations identified in primary multiple myeloma samples by whole exome sequencing
Excel file: Bohl et al supplemental table S8+9

Supplemental Table S9
List of genes included in the CRISPR-Cas9 screens
Excel file: Bohl et al supplemental table S8+9

Supplemental Table S10
Frequency of aberrations (%)in published multiple myeloma sequencing studies (blank squares = no data provided)

Mutation Frequency (%)	**Newly diagnosed**			**Relapsed/ Refractory**		
Study	Lohr et al., Cancer Cell 2011	Walker et al., JCO 2015	Weinhold et al., Blood 2016	Weinhold et al., Blood 2016	Chavan et al., BCJ 2017	Kortuem et al., Blood 2016
Analysis	Mutation	Mutation	Mutation/ Deletion	Mutation/ Deletion	Mutation/ Deletion	Mutation
Patients N	203	463	33	33	323	50
TP53	8	3	20	44	21	26
VPS35	0.5					
NDUFA10	0					
CHD8	0.5					
CRBN	0.5					12
CUL4B	0.5					6
DDB1	0					
ZNF236	1					
PCDHA5	3					
ANKMY2	0					
PCDHGB4	1.5					
BIRC3	0.5				2	2
TRAF3	5	4	30	30	6	8
FANCA	1		3	9		
RAD54B	0					
ATM	4	4 (+ATR)			2.5	10
BRCC3	0					
SMARCD2	0					
SETD2	1.5		6	12		
RIC1	0					
NFKB2	0.5					8
FAM46C	12	5	36	33		12
LEMD3	0.5					
RB1	3.4	1.5	63	66	6	2
CDKN2C	0.5		15	21	6	2

Supplemental Table S11

List of all sgRNA z-scores for CRISPR-Cas9 screens
Excel file: Bohl et al supplemental table S11

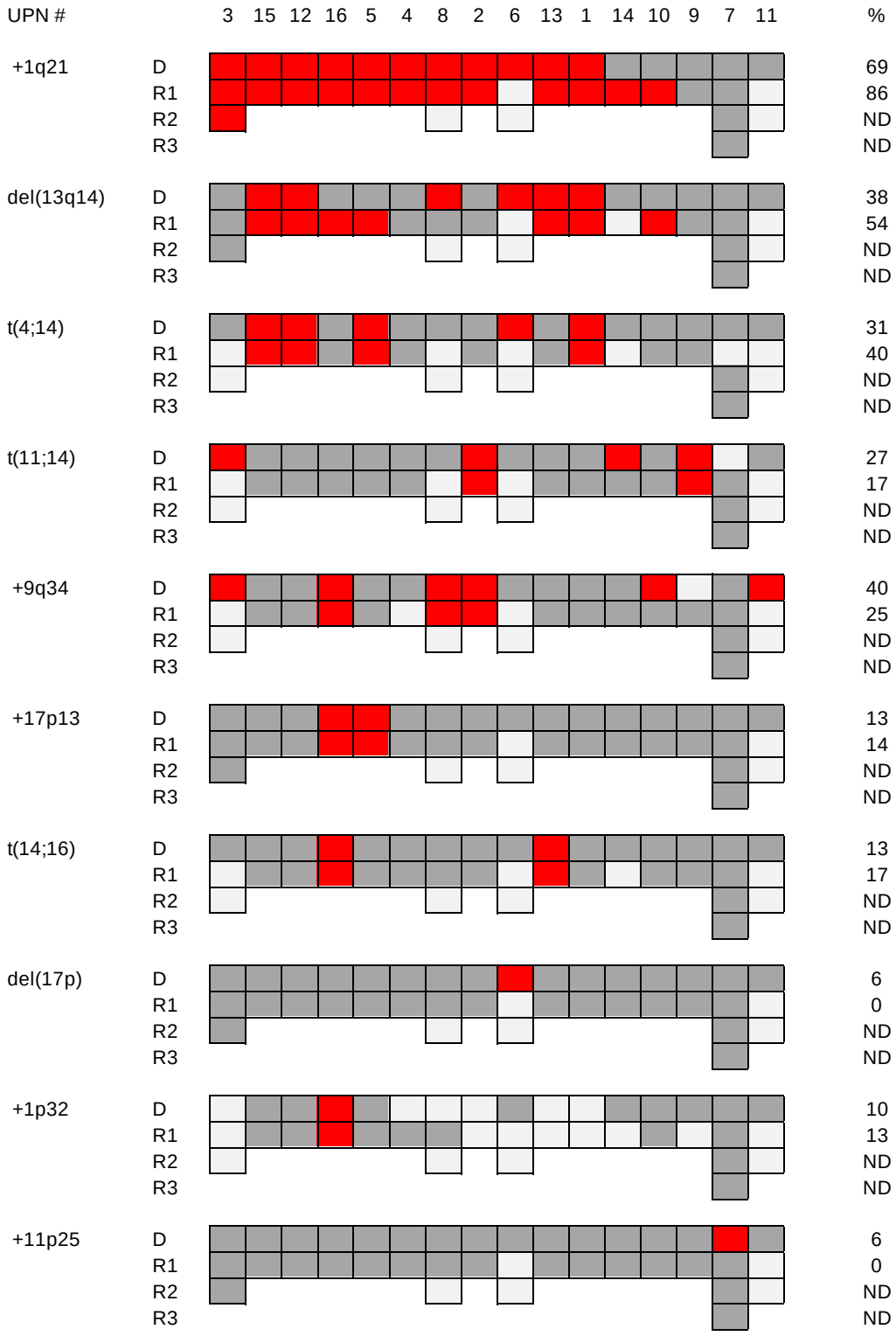
Supplemental Table S12

Primer pairs used for ultra-deep targeted sequencing of selected gene mutations in sequential samples.

UPN	Gene	Exon	Chr	Mut Start	Mut End	Forward primer	Reverse primer
1	ANKMY2	exon3	chr7	16666689	16666689	CCAATTGCCATCATTCCTTTCCA	GCGACATGGAGCCGATGTAA
3	DDB1	exon6	chr11	61093159	61093159	GGAGCAAGGTGAGGACAAGG	GACCCAGAGTGCTGAGCATT
7	RAD54B	exon4	chr8	95416375	95416375	AGTGTACCCTCAGGACTCAT	ACTGTTTCCCTCTTGTGGATGT
8	TP53	exon6	chr17	7578268	7578268	CAACCACCCTTAACCCCTCC	CCAGGCCTCTGATTCCTCAC
8	TP53	exon7	chr17	7577538	7577538	AAATCGGTAAGAGGTGGGCC	TGGCTCTGACTGTACCACCA
8	TP53	exon7	chr17	7577570	7577570	AAATCGGTAAGAGGTGGGCC	TGGCTCTGACTGTACCACCA
9	TP53	exon5	chr17	7578406	7578406	GGGCCAGACCTAAGAGCAAT	ACATGACGGAGGTTGTGAGG
14	TP53	exon6	chr17	7578253	7578253	CAACCACCCTTAACCCCTCC	CCAGGCCTCTGATTCCTCAC
14	TP53	exon5	chr17	7578442	7578442	GTGAGGAATCAGAGGCCTGG	GTGCAGCTGTGGGTTGATTC
14	TP53	exon8	chr17	7577121	7577121	GCAGTGCTAGGAAAGAGGCA	GCTTTGAGGTGCGTGTTTGT

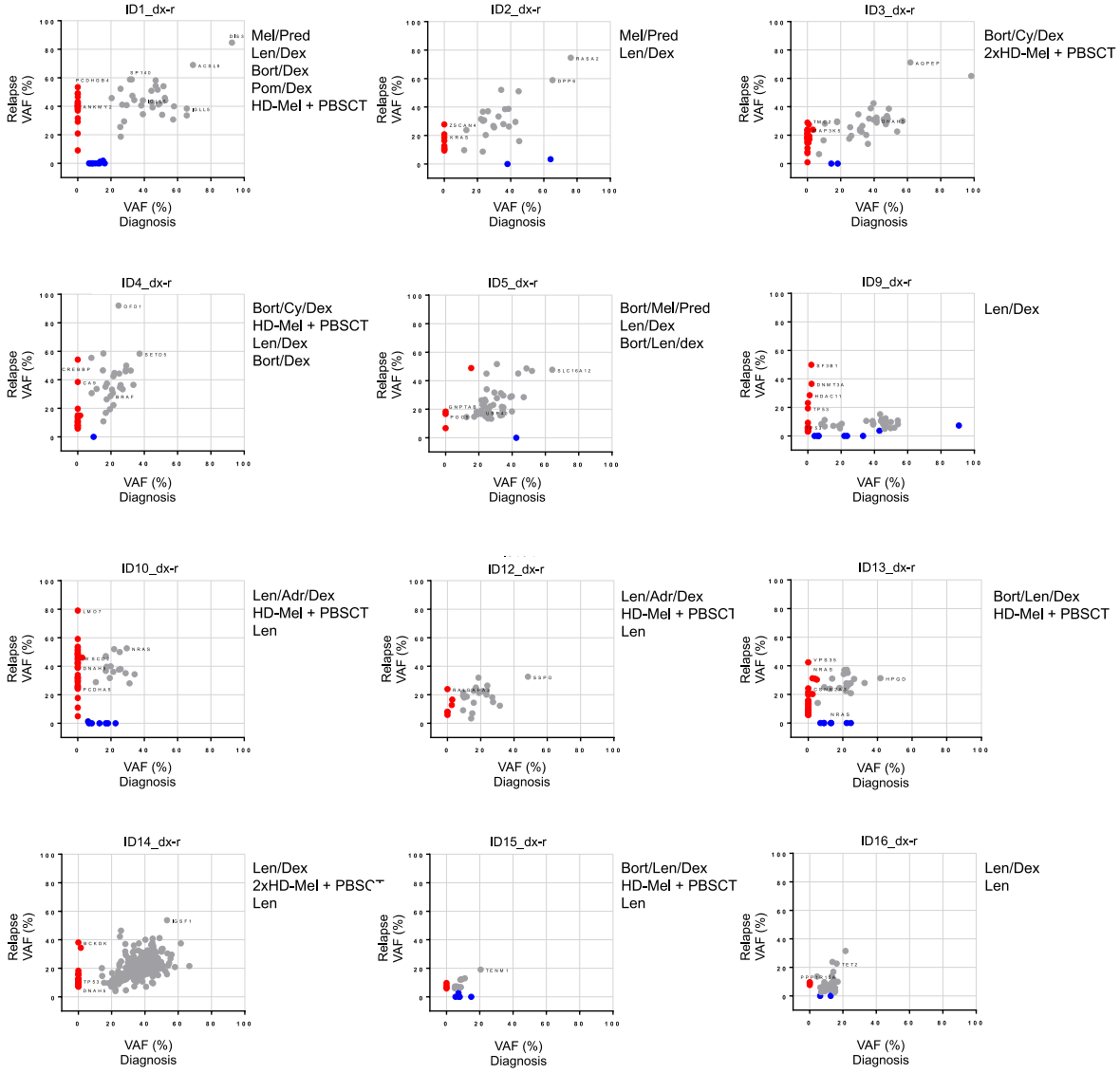
UPN=Unique patient number; Chr=Chromosome; Mut=Mutation

Supplemental Figures

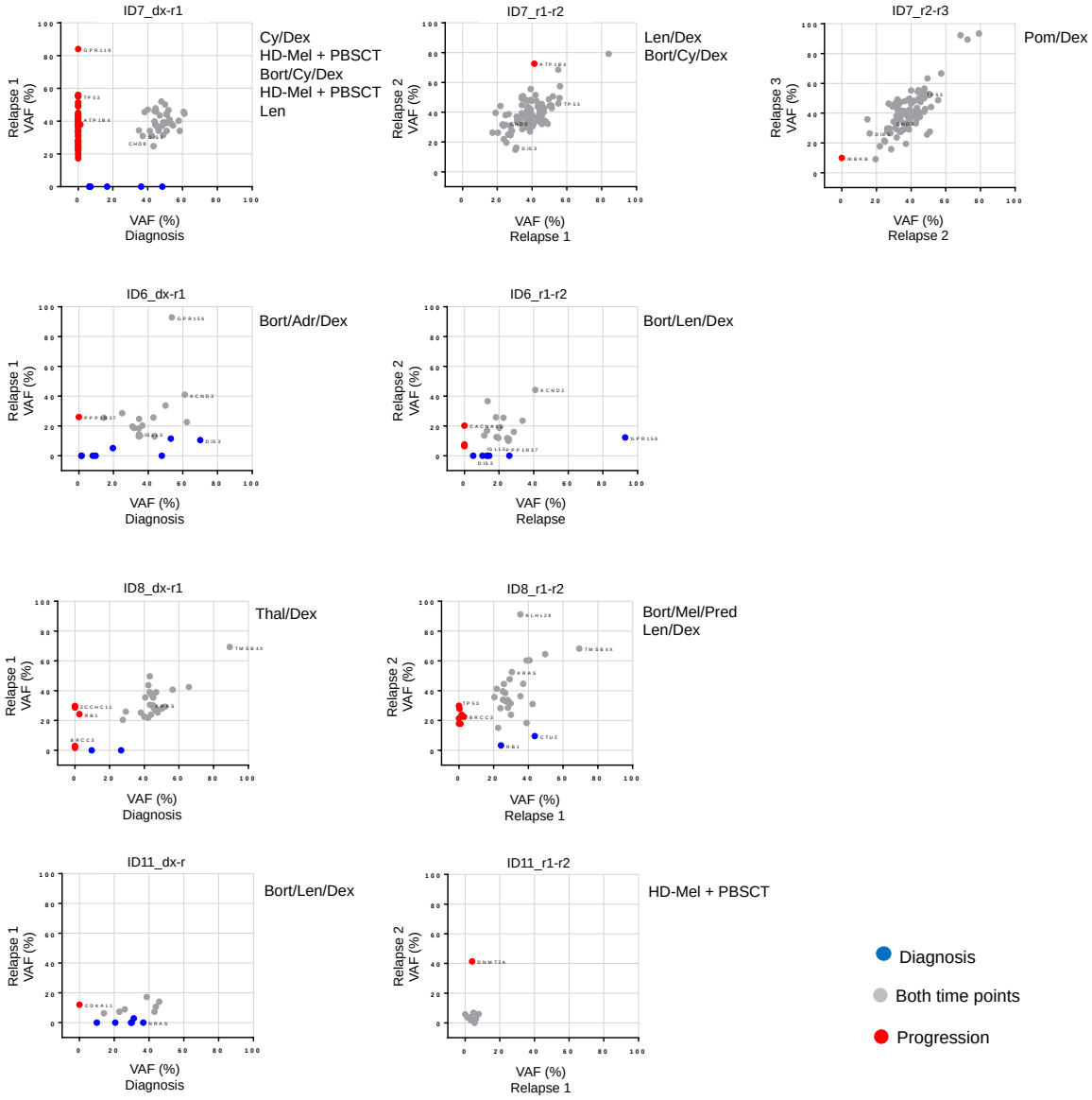


Supplemental Figure 1. Cytogenetic alterations as detected by FISH present at the time of diagnosis and at disease progression in multiple myeloma patients (n=16). Each column represents one patient (UPN=unique patient number). The percentage of each alteration at the respective time point is given right to the columns. Red=Genetic alteration present, dark grey=cytogenetic alteration not detected, light grey=information not available, ND=not determined.

A

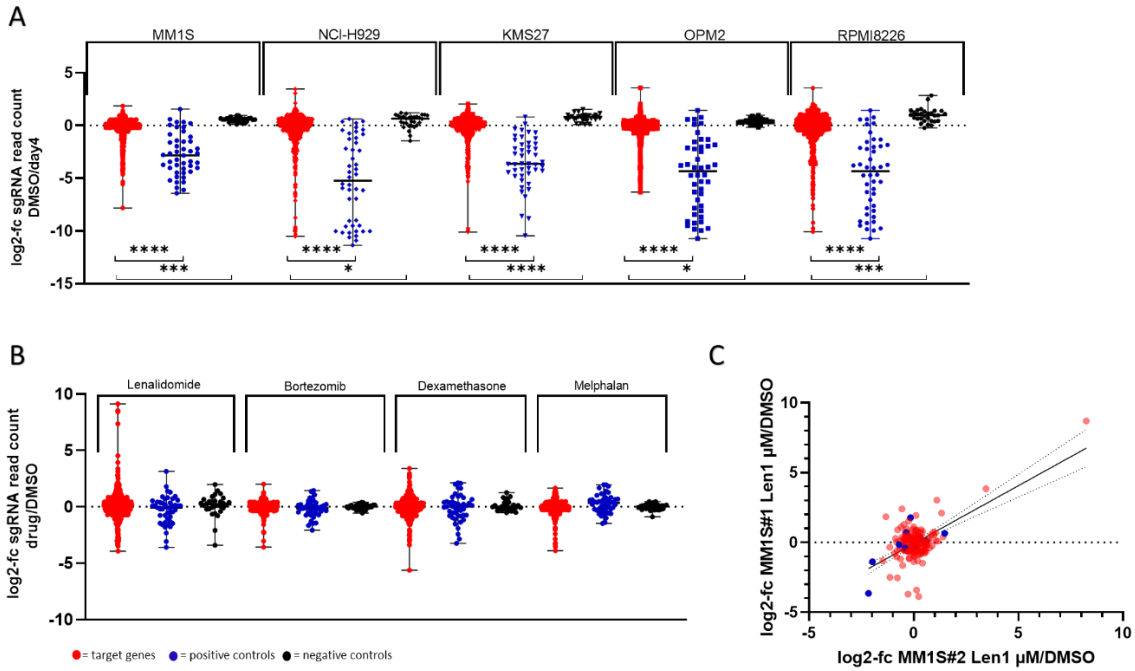

ID1_dx-r
Mel/Pred
Len/Dex
Bort/Dex
Pom/Dex
HD-Mel + PBSCT
ID2_dx-r
Mel/Pred
Len/Dex
ID3_dx-r
Bort/Cy/Dex
2xHD-Mel + PBSCT
ID4_dx-r
Bort/Cy/Dex
HD-Mel + PBSCT
Len/Dex
Bort/Dex
ID5_dx-r
Bort/Mel/Pred
Len/Dex
Bort/Len/dex
ID9_dx-r
Len/Dex
ID10_dx-r
Len/Adr/Dex
HD-Mel + PBSCT
Len
ID12_dx-r
Len/Adr/Dex
HD-Mel + PBSCT
Len
ID13_dx-r
Bort/Len/Dex
HD-Mel + PBSCT
ID14_dx-r
Len/Dex
2xHD-Mel + PBSCT
Len
ID15_dx-r
Bort/Len/Dex
HD-Mel + PBSCT
Len
ID16_dx-r
Len/Dex
Len
Relapse VAF (%)
VAF (%) Diagnosis

B

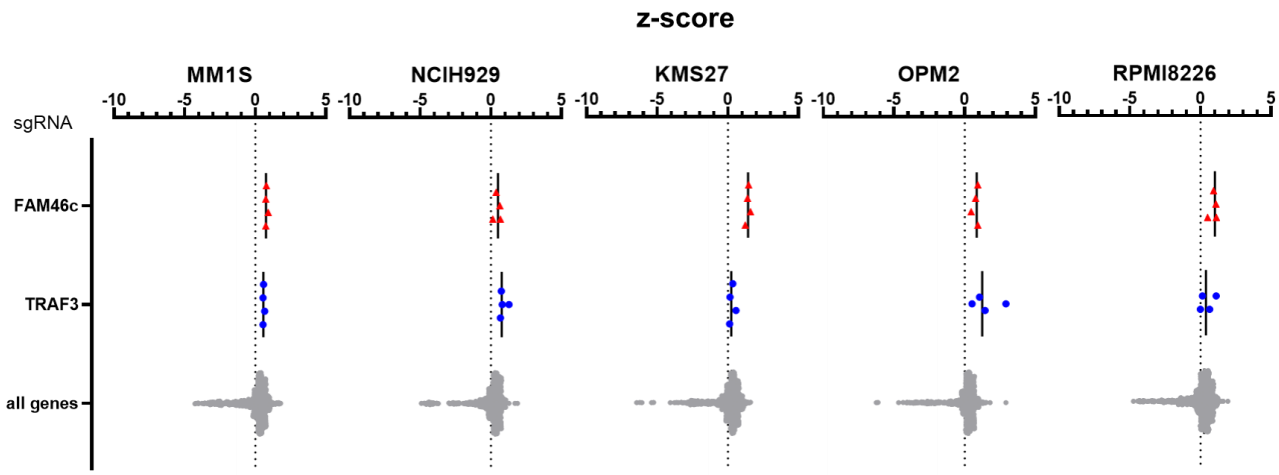


dx=diagnosis; r=relapse; VAF=variant allele frequency; Adr=Adriamycin; PBSCT=autologous stem cell transplantation; Bort=Bortezomib; Cy=Cyclophosphamid; Dex=dexamethasone; HD=high-dose; transplantation; Len=lenalidomid; Mel=melphalan; Pom=pomalidomide; Thal=thalidomide; Pred=prednisolone

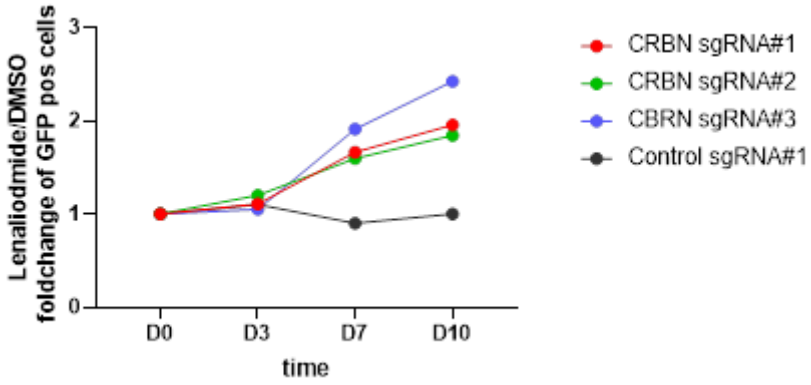
Supplemental Figure 2. Mutations present at diagnosis and disease progression. Each graph represents one patient (A) or serial progression sample of one patient (B) as indicated on the top of each graph. The variant allele frequency (VAFs) of each mutation at diagnosis (x-axis) and relapse (y-axis) are represented as dots. Blue indicates that the mutation was present only at diagnosis or with a higher VAF, red indicates that the mutation was only present at relapse or with a higher VAF. Mutations with similar VAFs at both time point are color-coded in grey. Treatment regimens between both time points are given on the right of the graphs.



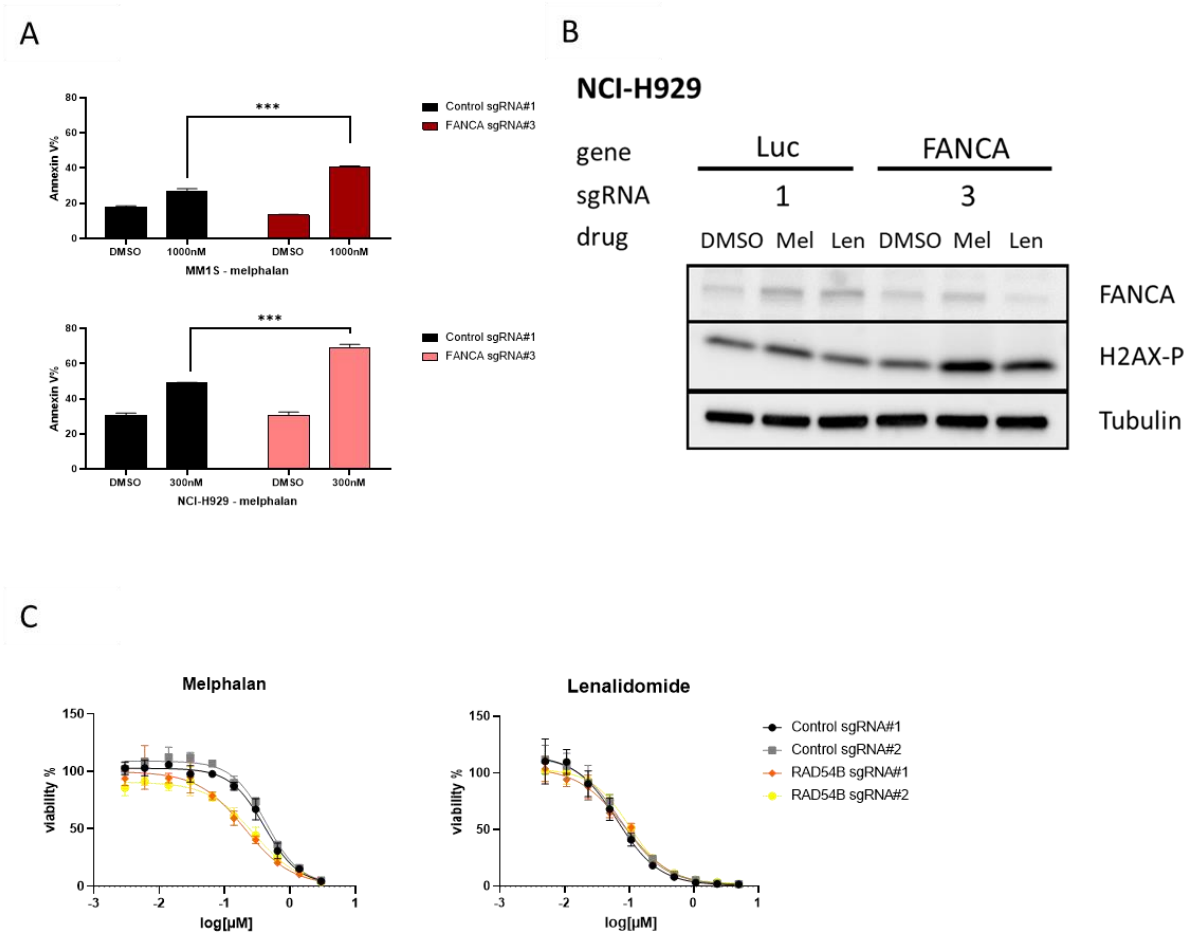
Supplemental Figure 3: **Overview of CRISPR screens**.
Five multiple myeloma cell lines (MM1S,NCI-H929, KMS27, OPM2, RPMI8226) were transduced with the lentiviral pooled custom sgRNA libraries and treated with either DMSO as control, lenalidomide, bortezomib, melphalan or dexamethasone, respectively, and tracked via flow cytometry every 3-4 days. After 14 passages or a cell viability lower than 10%,cells were harvested and analyzed for sgRNA abundance. (A) log2-fold change of sgRNAs of target genes, positive controls and negative controls in each cell line at the end of DMSO control screen. (B) log2-fold change for each sgRNA for each drug in CRISPR screens in MM1S cells. (C) Correlation of the gene log2-fold change of two biological replicates for the lenalidomide CRISPR resistance screen in MM1S cells ($p<0.001$, Pearson $r= 0.60$).



Supplemental Figure 4: **Effect of *FAM46c* and *TRAF3* inactivation on cell proliferation**
SgRNAs targeting the two tumor suppressor genes *FAM46c* (red) and *TRAF3* (blue) were enirched in the DMSO control preferentially in celllines not carrying the respective mutation.



Supplemental Figure 5: Validation of individual sgRNAs targeting *CRBN*
MM1S-Cas9 cells were infected with lentiviral vectors expressing luciferase control- or *CRBN*-specific sgRNAs and GFP and treated with 1µM lenalidomide. Flow cytometry was performed to determine the fraction of transduced cells.

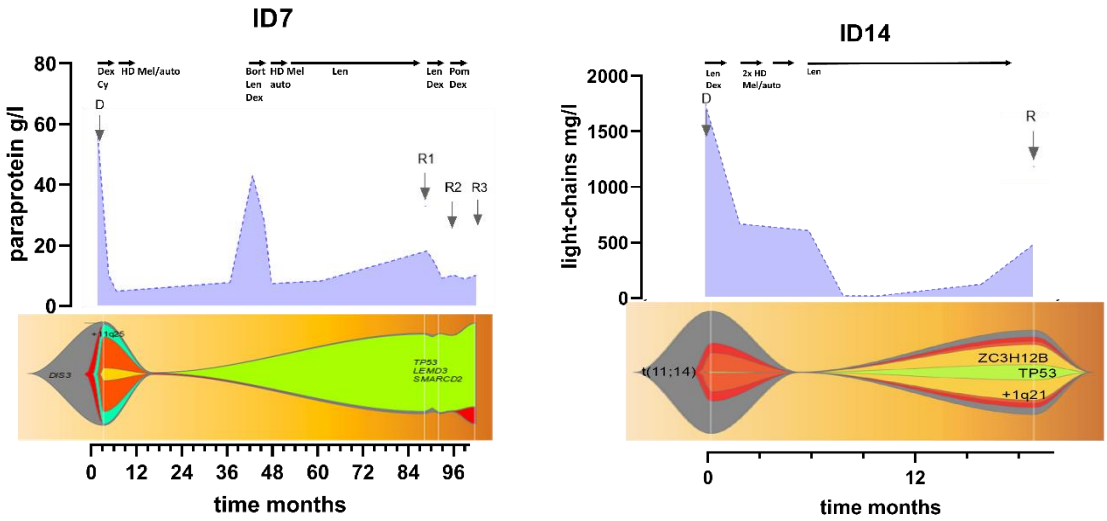


Supplemental Figure 6: **Effects of FANCA and RAD54b inactivation on melphalan activity**

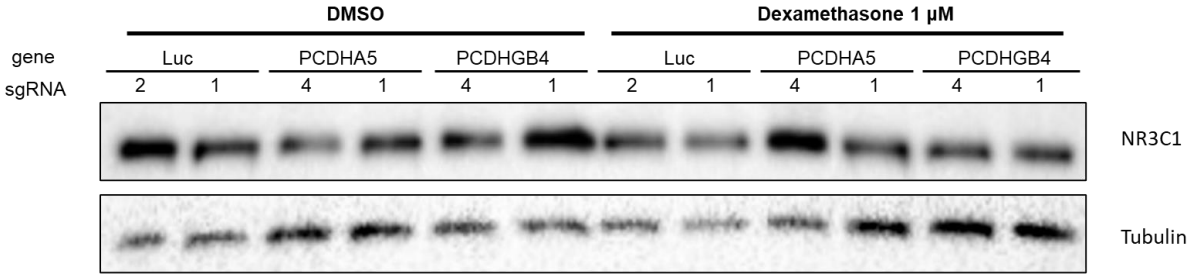
(A) MM1S- and NCIH 929-Cas9 cells infected with lentiviral vectors expressing *luciferase* contro-l or *FANCA*-specific sgRNAs were treated with DMSO or melphalan for 4 days. Cells were stained with Annexin V and Hoechst followed by flow cytometry analysis. (***=p<0.0001)

(B) MM1S and NCI-H929-Cas9 cells expressing sgRNAs against *luciferase*- or *FANCA*- were treated with DMSO, 1µM Melphalan or 1µM Lenalidomide for 36hours and analyzed for increased DNA damage using Phospho-H2A.X mAb.

(C) MM1S-Cas9 cells infected with *luciferase* control- (grey) or *RAD54b*-(yellow/orange) specific sgRNAs were treated with DMSO, melphalan or lenalidomide for 7 days, with dosing at day 0 and day 4. Dose response curves show cell viability normalized to DMSO treatment.



Supplemental Figure 7. Clonal evolution in patient #7 and #14.
Courses of paraprotein (purple graph, top) and fishplots showing the clonal evolution of mutations between diagnosis and disease progression in two patients with outgrowth of *TP53* mutations after melphalan treatment. Treatment regimens are given on the top of the plots. Arrows indicate the time of sample collection. (D=Diagnosis, R=Relapse; Bort=Bortezomib, Dex=Dexamethasone, Cy=Cyclophosphamide, HD Mel=High-dose melphalan, auto=autologous stem cell transplantation, Len=Lenalidomide, Pom=Pomalidomide).



Supplemental Figure 8: **Effect of *PCDHA5* and *PCDHGB4* inactivation on protein expression of the glucocorticoid receptor NR3C1**
Protein levels of NR3C1 analyzed by western blot in MM1S cells with CRISPR-Cas9 mediated inactivation of *PCDHA5* and *PCDHGB4* with and without 1µM dexamethasone treatment. Protein lysates were harvested and blotted for indicated proteins. Data are representative of 2 experiments.