Microhomology-mediated end joining drives complex rearrangements and overexpression of *MYC* and *PVT1* in multiple myeloma

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Authors

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Contents

Supplementary Methods

Patient Samples and Next Generation Sequencing

Total of 1267 NDMM were included in this study after informed consent. Plasma cell were isolated from bone marrow by magnetic-activated cell sorting using $CD138^+$ marker, AutoMACS Pro (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) or Robosep (STEMCELL Technologies, Vancouver, Canada). DNA from peripheral blood was used as a control sample for each patient to exclude germline variants. Three paired-end read sequencing platforms were combined without overlapping patients. Overall summary of methods, number of patients and external datasets are demonstrated **in Supplementary Figure 1**. Patients' characteristics are summarized in **Supplementary Table 1** and *MYC* region capture is illustrated in **Supplementary Figure 2**.

a. Targeted sequencing (n=100): DNA was isolated using AllPrep DNA/RNA Kit (Qiagen, Hilden, Germany). Total of 50 ng of DNA was enzymatic fragmented and library was prepared using KAPA HyperPlus Kit (Kapa Biosystems, Wilmington, MA, USA) and SeqCap EZ Kit (Roche NimbleGen, Basel, Switzerland). A total of 4.8 Mb was targeted and designed in two parts. First, 4.2 Mb covering *IGH*, *IGK*, *IGL* and *MYC* genes focusing on translocations and chromosomal structure abnormalities. Second, 0.6 Mb covering exonic regions of 127 MM-specific genes and 27 chromosome regions for gene mutations and copy-number abnormalities analysis. Hybridization reactions were performed separately for each targeted-enrichment part and samples were finally combined at appropriate ratio to get required depth for chromosome structure abnormalities (~100x) and gene mutations (~250x) part. HiSeq 2500 (Illumina, San Diego, CA, USA) was used for sequencing. The DNA quality and quantity were measured by Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and/or 2200 Tapestation (Agilent Technologies, Santa Clara, CA, USA). With focus on *MYC*, 4.5 Mb region (chr8:126.3–130.8 Mb) surrounding the gene was targeted with 83.1% capture. *MYC* expression level was defined in 98 patients by gene expression profiling using U133Plus2.0 microarray platform (Affymetrix, Santa Clara, CA) as previously described.[1]

b. Whole exome sequencing (n=461): A previous published dataset of patients with custom-enriched exome sequencing was used with detailed description of the protocol.[2] Briefly, DNA was isolated using AllPrep DNA/RNA Kit (Qiagen, Hilden, Germany). A total of 200 ng of DNA was fragmented using Covaris E-Series. NEBNext DNA library prep master mix set for Illumina (New England Biolabs, Ipswich, MA, USA) was used for library preparation. Exome enrichment was performed by custom designed RNA baits (SureSelect Human All Exon V5, Agilent Technologies; enriched for *IGH*, *IGK*, *IGL* and *MYC* region capture). Samples were sequenced using a HiSeq 2000 (Illumina, San Diego, CA, USA). The DNA quality and quantity were measured by Pico-green (Thermo Fisher Scientific, Waltham, MA, USA) and/or 2200 Tapestation (Agilent Technologies, Santa Clara, CA, USA). A region 2.3 Mb (chr8:127.5–129.8 Mb) surrounding *MYC* with 100% capture was targeted.

c. Genome sequencing (n=706): Dataset of patients was provided by Multiple Myeloma Research Foundation CoMMpass study and it is composed of patients with varying treatment strategies including bortezomib or carfilzomib-based regimens that may have been combined with IMiDs. Long-insert-based genome sequencing data was used for *MYC* translocation and chromosomal abnormalities study of the region in size of 5.0 Mb surrounding *MYC* (chr8:126.0–131.0 Mb). Exome sequencing available in 703 of 706 patients was used for NS-SNVs analysis. Expression of genes was quantified by RNA-Sequencing available in 571 of 706 patients.

Data Analysis

Data analysis was performed as described previously, with minor differences between sequencing modalities.[3] Briefly, FASTQ files from targeted sequencing (TS), whole exome sequencing (WES) and whole genome sequencing (WGS) were aligned to the human genome assembly GRCh37 by BWA-MEM (v0.7.12). Variants were called using MuTect2 and Strelka (v1.0.14 in TS, v1.0.15 in WES and WGS), filtered using fpfilter (https://github.com/ckandoth/variant-filter) in TS and a custom filter described elsewhere in WES and WGS.[3] A minimum 10% VAF filter was used for indels. Variant annotation was provided by Variant Effect Predictor (v85) in TS or Oncotator (v1.9.0) in WES and WGS.

Intra- and inter-chromosomal rearrangements were called using Manta[4] (v0.29.6 in WES and v1.0.1 in WES and WGS) with default settings and the exome flag specified for TS and WES samples. Copy-number alterations were determined in TS by normalized tumor/germline depth ratio supported by allele ratio changes in individual heterozygous SNP loci. All *MYC*-region-associated chromosomal breakpoints and copy number abnormalities were manually inspected. Cases with more than five chromosomes involved in the translocation (n=14) or more than five intra-chromosomal rearrangements at 8q24 (n=18) were considered as abnormal, but for high inter- or intra-chromosomal complexity they were excluded from detailed analysis. *MYC* region annotations for the CoMMpass and UK datasets are detailed in a previous publication.[3]

Manta was used to evaluate sequence homology between breakpoints in WGS data. All passed translocation events were filtered to only include classic *IGH* or *MYC* translocations. All events with the IMPRECISE flag set in Manta were filtered out. The homology length (HOMLEN) parameter was extracted from the INFO field in the Manta VCF. Fields without a HOMLEN parameter were set to zero. To ensure viability of Manta homology detection we manually verified randomly selected samples (see **Supplementary Alignments**). Events with only one nucleotide homology between breakpoints were not considered for analysis due to the fact that those could be simply due to chance. Finally, *IGH* and *MYC* events with no sequence homology were compared to *IGH* and *MYC* events with two or more nucleotide homology using Fisher's exact test.

RNA-Sequencing data was aligned to the human genome assembly GRCh38 with gene-transcripts quantification processing by Star (v2.5.1b) and Salmon (v0.6.0) algorithms. The read counts per gene from Salmon were read into R and using the DESeq2 (v1.20.0) R library, normalized across samples and the $\log_2$ expression calculated. A total of 526 patients with available RNA-Sequencing data and hyperdiploidy status were analyzed for a *MYC* signature using limma R package. Genes with more than 0.5% of zero values were excluded from the analysis, remaining genes were adjusted for hyperdiploidy status and filtered by FDR≤0.05 and fold change >=1.8. Threshold $\log_2$=13.0 for *MYC*-expression-based signatures was discriminated by receiver operating characteristics (ROC) analysis (AUC=0.85) as intersection between the

highest sensitivity (0.75) and specificity (0.82) to predict abnormal genomic profiles. Gene enrichment was performed by Gene Ontology Consortium analysis with Fisher's test with FDR multiple test correction (P≤0.05).

External Datasets

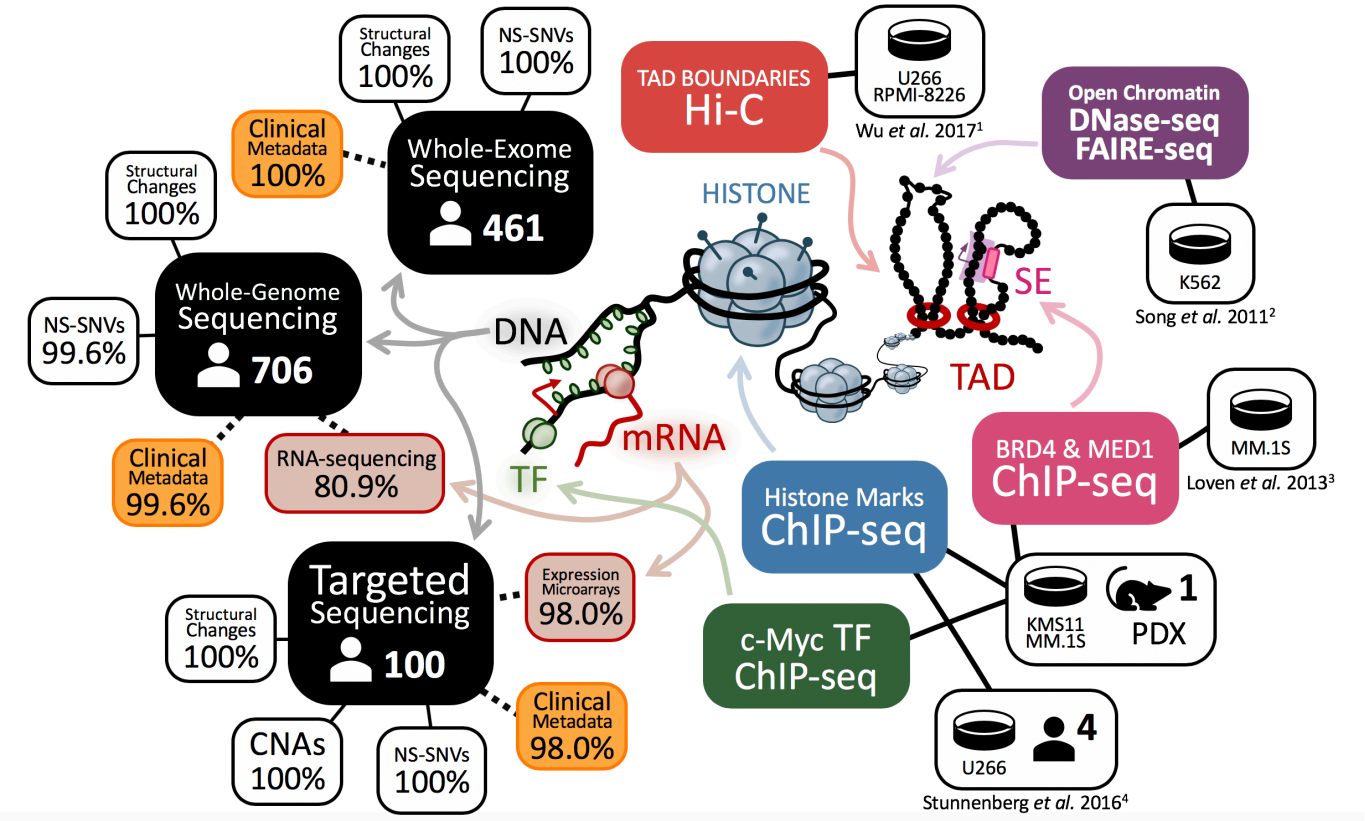
Genomic annotations of breakpoint regions were taken from previously published sources. Super-enhancer sites were taken from the MM.1S myeloma cell line.[5] TADs were taken from the MM cell lines U266 and RPMI-8226.[6] Chromatin marks were taken from the MM cell line U266 and four myeloma cell samples.[7, 8] Open chromatin was identified by a combination of DNase-Seq and FAIRE-Seq in cell line K562.[9]

References

1. Zhan F, Hardin J, Kordsmeier B, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. Blood. 2002;99(5):1745-1757.
2. Walker BA, Boyle EM, Wardell CP, et al. Mutational Spectrum, Copy Number Changes, and Outcome: Results of a Sequencing Study of Patients With Newly Diagnosed Myeloma. J Clin Oncol. 2015;33(33):3911-3920.
3. Walker BA, Mavrommatis K, Wardell CP, et al. Identification of novel mutational drivers reveals oncogene dependencies in multiple myeloma. Blood. 2018;
4. Chen X, Schulz-Trieglaff O, Shaw R, et al. Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. Bioinformatics. 2016;32(8):1220-1222.
5. Loven J, Hoke HA, Lin CY, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell. 2013;153(2):320-334.
6. Wu P, Li T, Li R, et al. 3D genome of multiple myeloma reveals spatial genome disorganization associated with copy number variations. Nat Commun. 2017;8(1):1937.
7. Stunnenberg HG, International Human Epigenome C, Hirst M. The International Human Epigenome Consortium: A Blueprint for Scientific Collaboration and Discovery. Cell. 2016;167(7):1897.
8. Adams D, Altucci L, Antonarakis SE, et al. BLUEPRINT to decode the epigenetic signature written in blood. Nat Biotechnol. 2012;30(3):224-226.
9. Song L, Zhang Z, Grasfeder LL, et al. Open chromatin defined by DNaseI and FAIRE identifies regulatory elements that shape cell-type identity. Genome Res. 2011;21(10):1757-1767.

Supplementary Figures

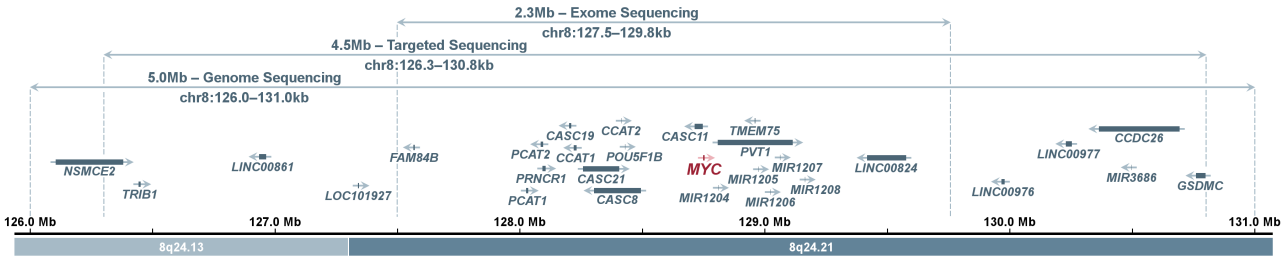
Supplementary Figure 1: Graphical overview of methods, internal and external datasets.



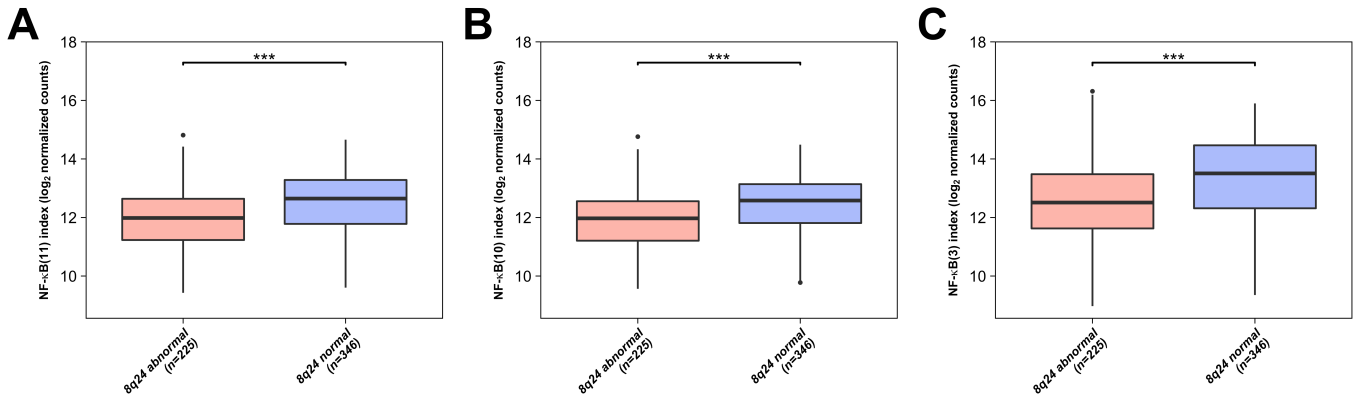
References:

1. Wu P, Li T, Li R, et al. 3D genome of multiple myeloma reveals spatial genome disorganization associated with copy number variations. Nat Commun. 2017;8(1):1937.
2. Song L, Zhang Z, Grasfeder LL, et al. Open chromatin defined by DNaseI and FAIRE identifies regulatory elements that shape cell-type identity. Genome Res. 2011;21(10):1757-1767.
3. Loven J, Hoke HA, Lin CY, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell. 2013;153(2):320-334.
4. Stunnenberg HG, International Human Epigenome C, Hirst M. The International Human Epigenome Consortium: A Blueprint for Scientific Collaboration and Discovery. Cell. 2016;167(7):1897.

Supplementary Figure 2: Illustration of the studied *MYC* region at 8q24 in each dataset. 461 cases with custom-enriched whole exome sequencing (up), 100 cases with targeted sequencing (middle), 706 cases with whole genome sequencing (down).



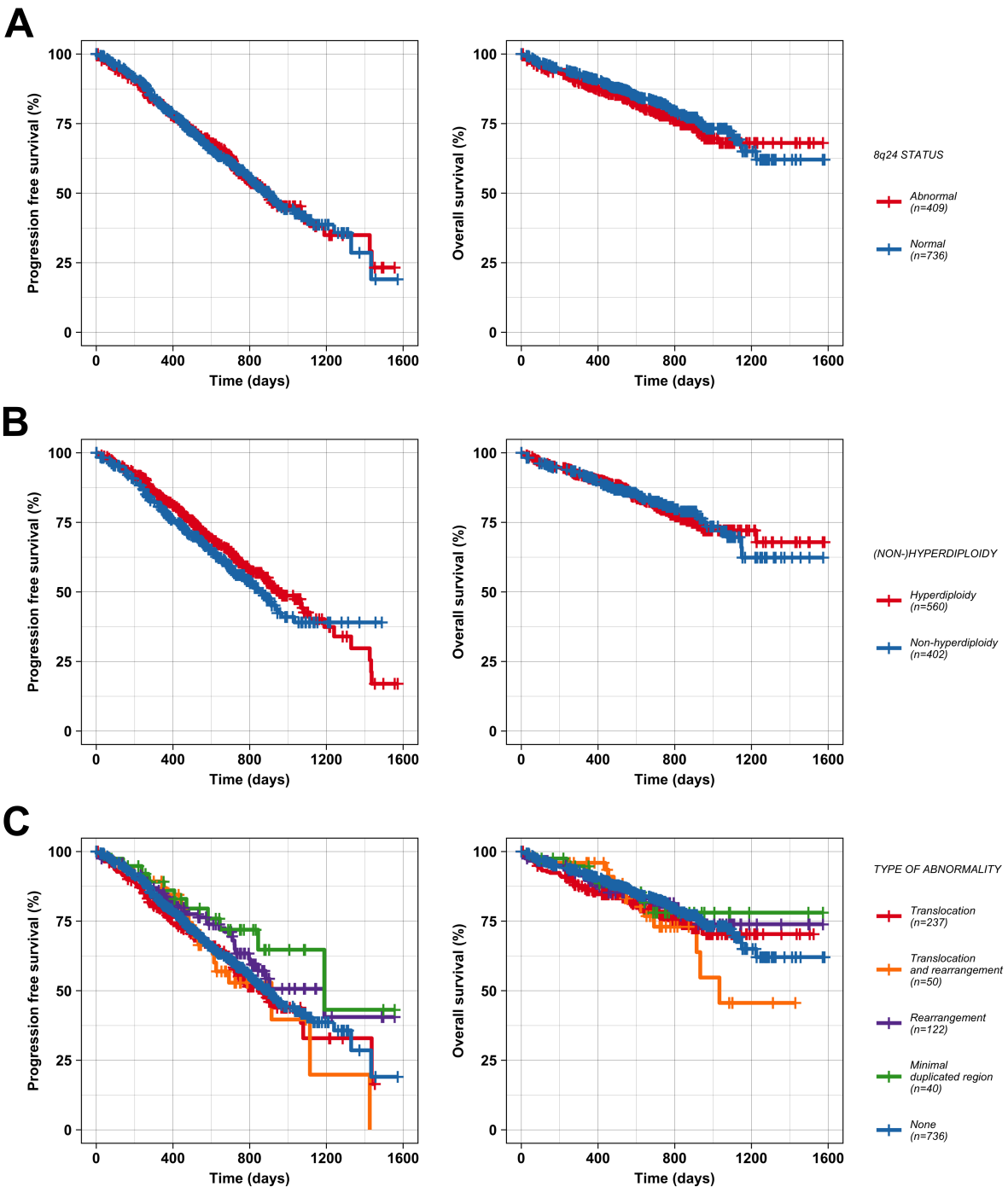
Supplementary Figure 3: Association of 8q24 abnormalities and NF-κB pathway activation. NF-κB pathways activation was defined as an average expression of the genes as follows: **(A)** NF-κB(11)[1] – *BIRC3*, *TNFAIP3*, *NFKB2*, *IL2RG*, *NFKBIE*, *RELB*, *NFKBIA*, *CD74*, *PLEK*, *MALT1*, *WNT10A*; **(B)** NF-κB(10)[2] – same as previous, excluding *BIRC3*; and **(C)** NF-κB(3)[2] – *TNFAIP3*, *IL2RG* and *BIRC3* (C). Expression was analyzed using RNA-sequencing. Statistically significant levels are as follows: ***P<0.001, **P<0.01 and *P<0.05.



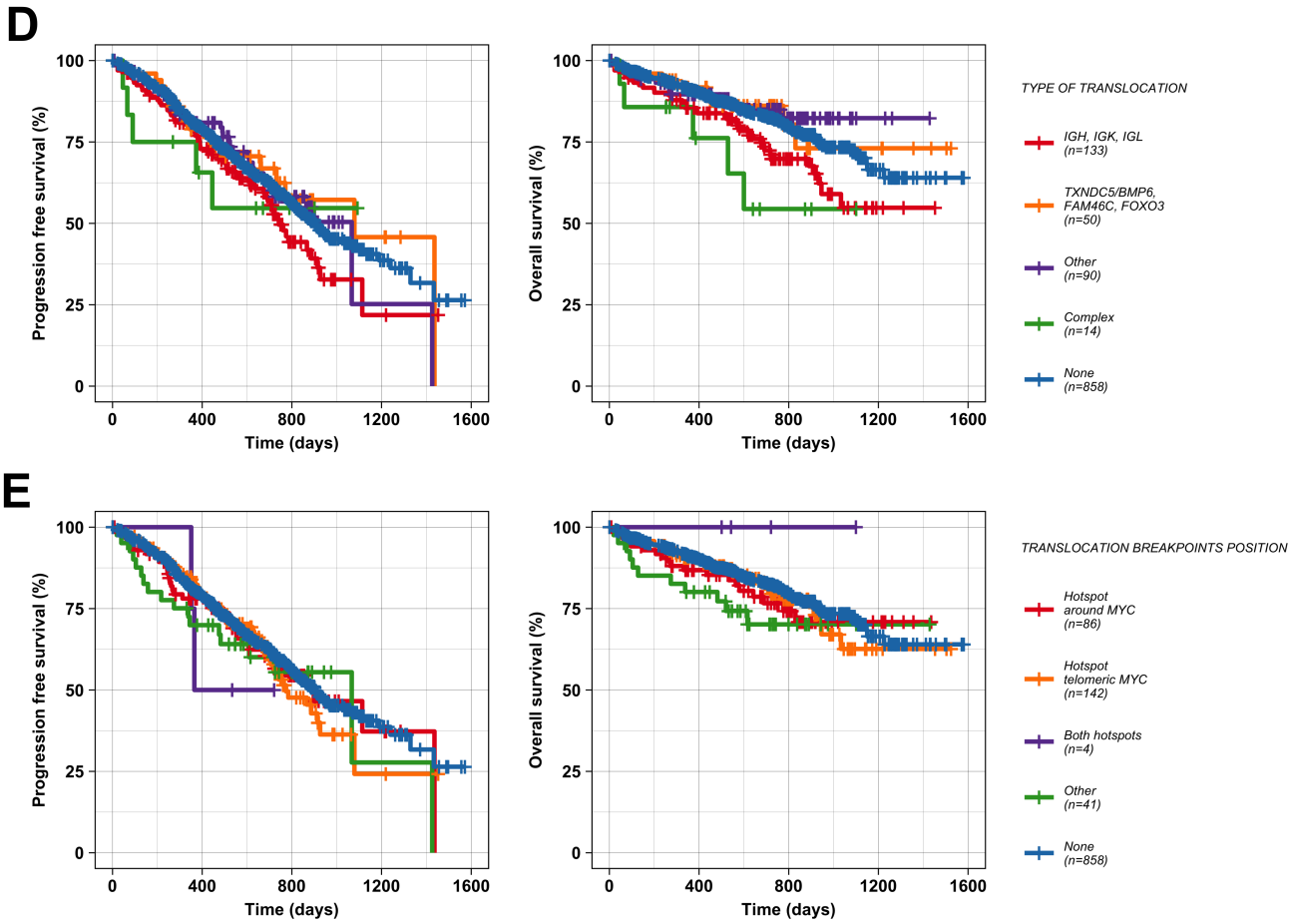
References:

1. Annunziata CM, Davis RE, Demchenko Y, et al. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. Cancer Cell. 2007;12(2):115-130.
2. Demchenko YN, Glebov OK, Zingone A, Keats JJ, Bergsagel PL, Kuehl WM. Classical and/or alternative NF-kappaB pathway activation in multiple myeloma. Blood. 2010;115(17):3541-3552.

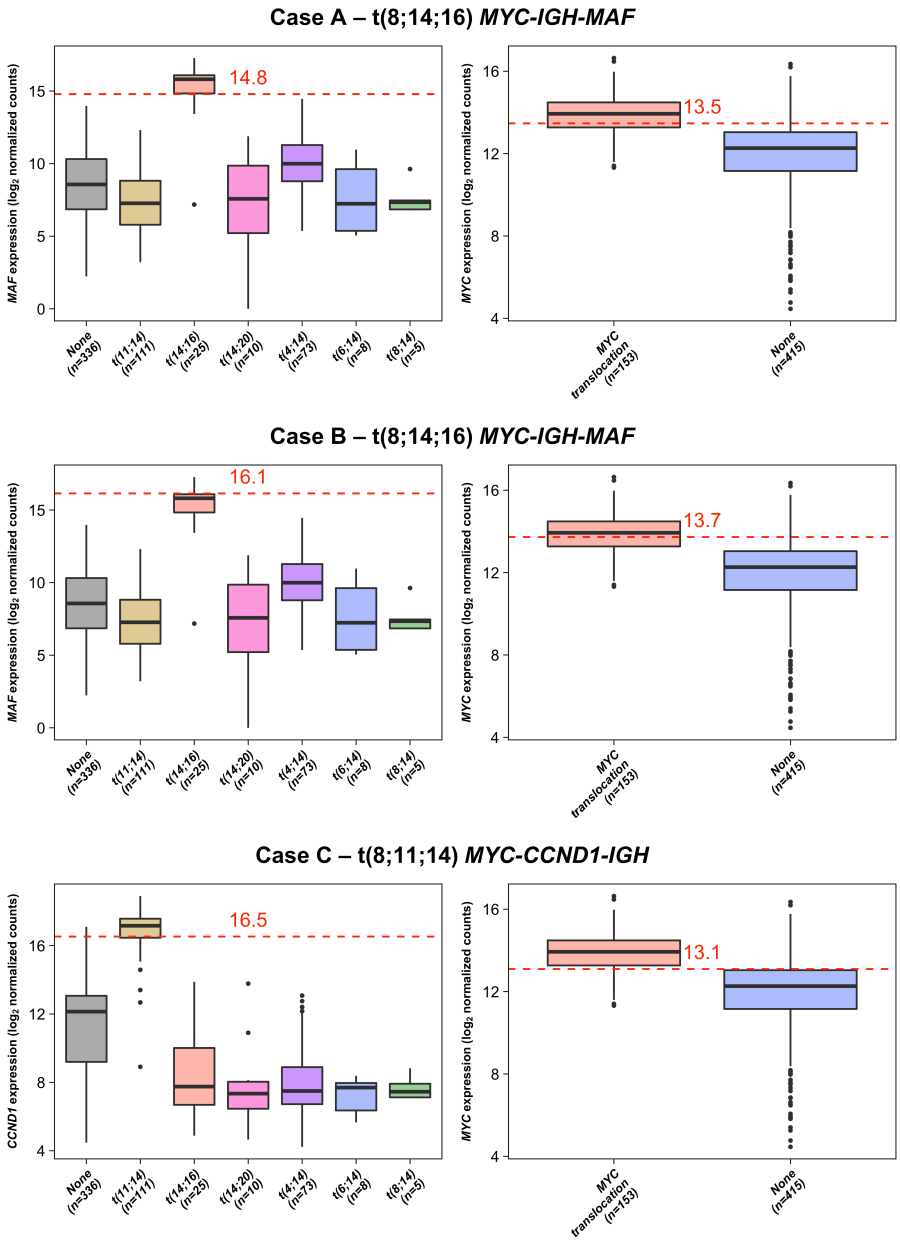
Supplementary Figure 4(A–C): Effect of 8q24 abnormalities on patients' outcome. (A) 8q24 abnormalities. **(B)** Hyperdiploidy status. **(C)** Type of 8q24 abnormality. Statistically significant levels are as follows: ***P<0.001, **P<0.01 and *P<0.05. No significant P was found.



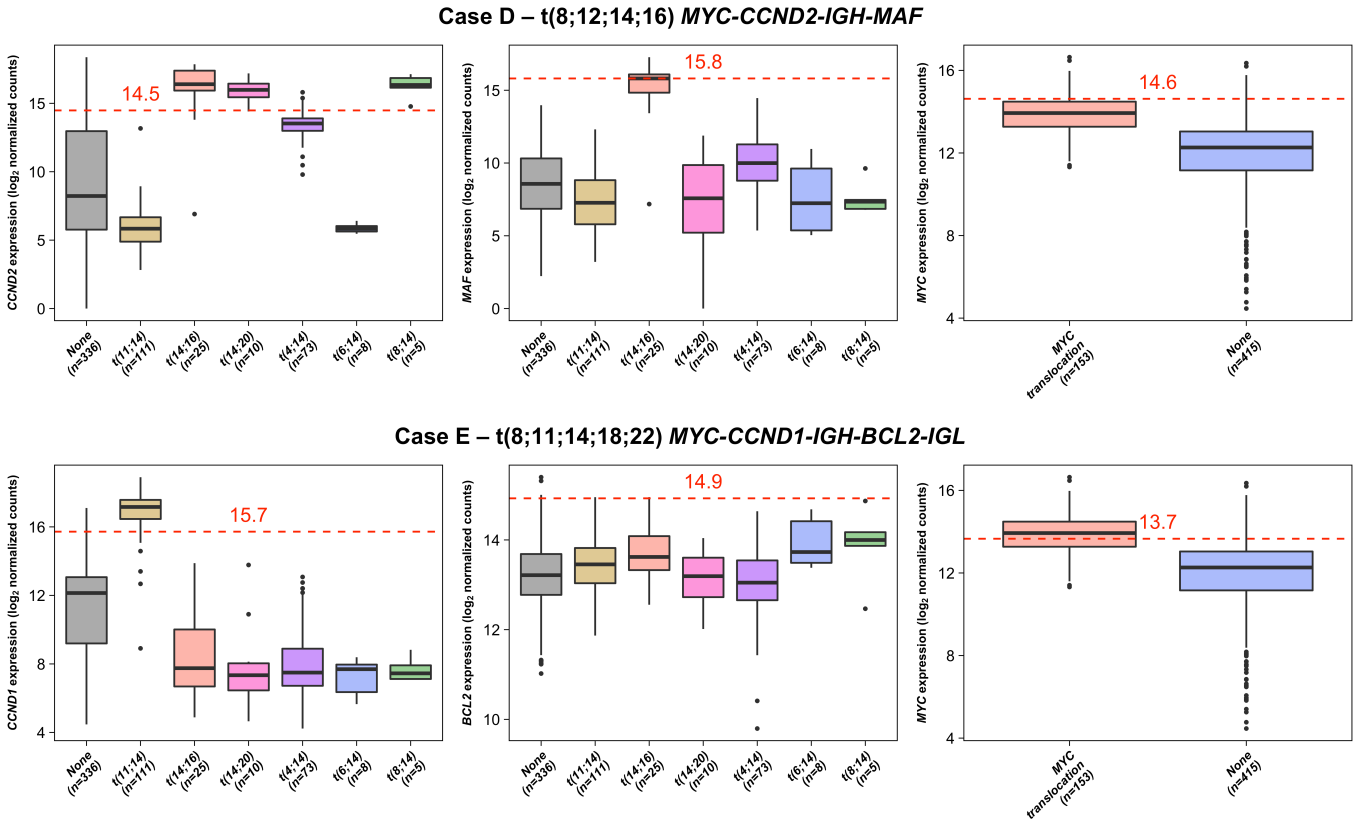
Supplementary Figure 4(D–E): Effect of 8q24 abnormalities on patients' outcome. (D) Translocation category. **(E)** Translocation breakpoint position. Statistically significant levels are as follows: ***P<0.001, **P<0.01 and *P<0.05. No significant P was found.



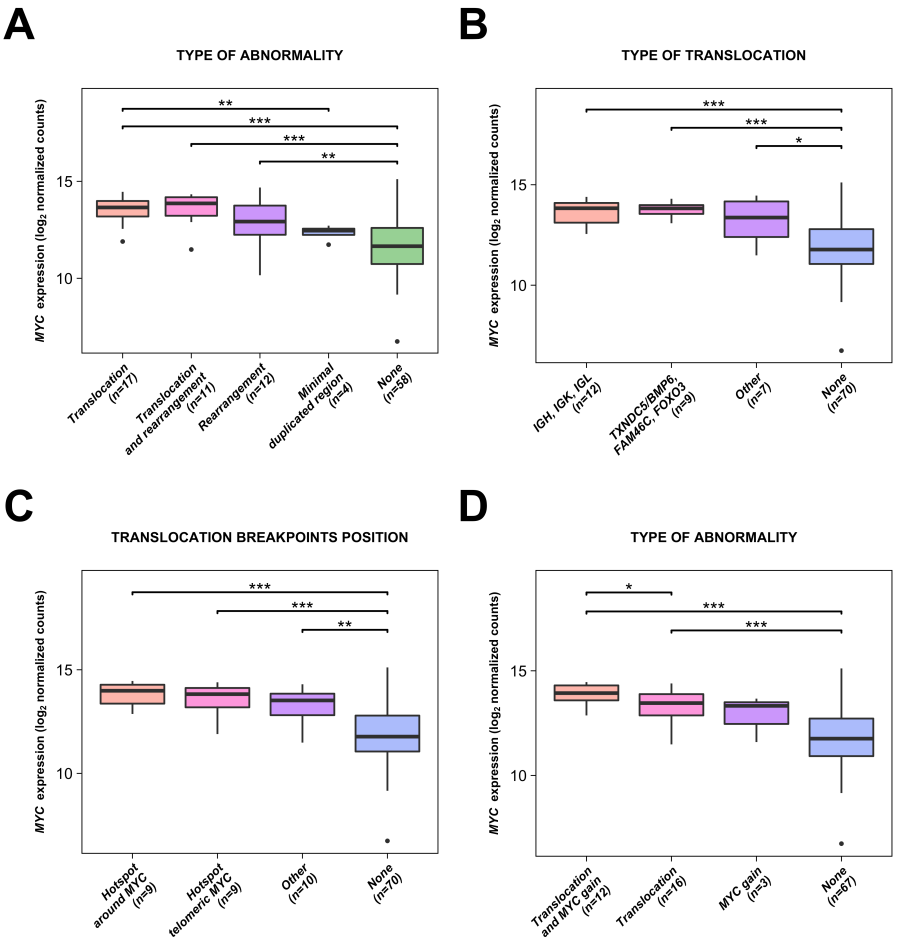
Supplementary Figure 5(A–C): Expression of oncogenes in complex translocations in five cases with available RNA-sequencing data. Box plots show expression distribution of the oncogene in specific *IGH* (left) and *MYC* (right) translocation groups. Red line determines a level of the oncogene expression in the case with complex translocation. Expression was analyzed using RNA-sequencing.



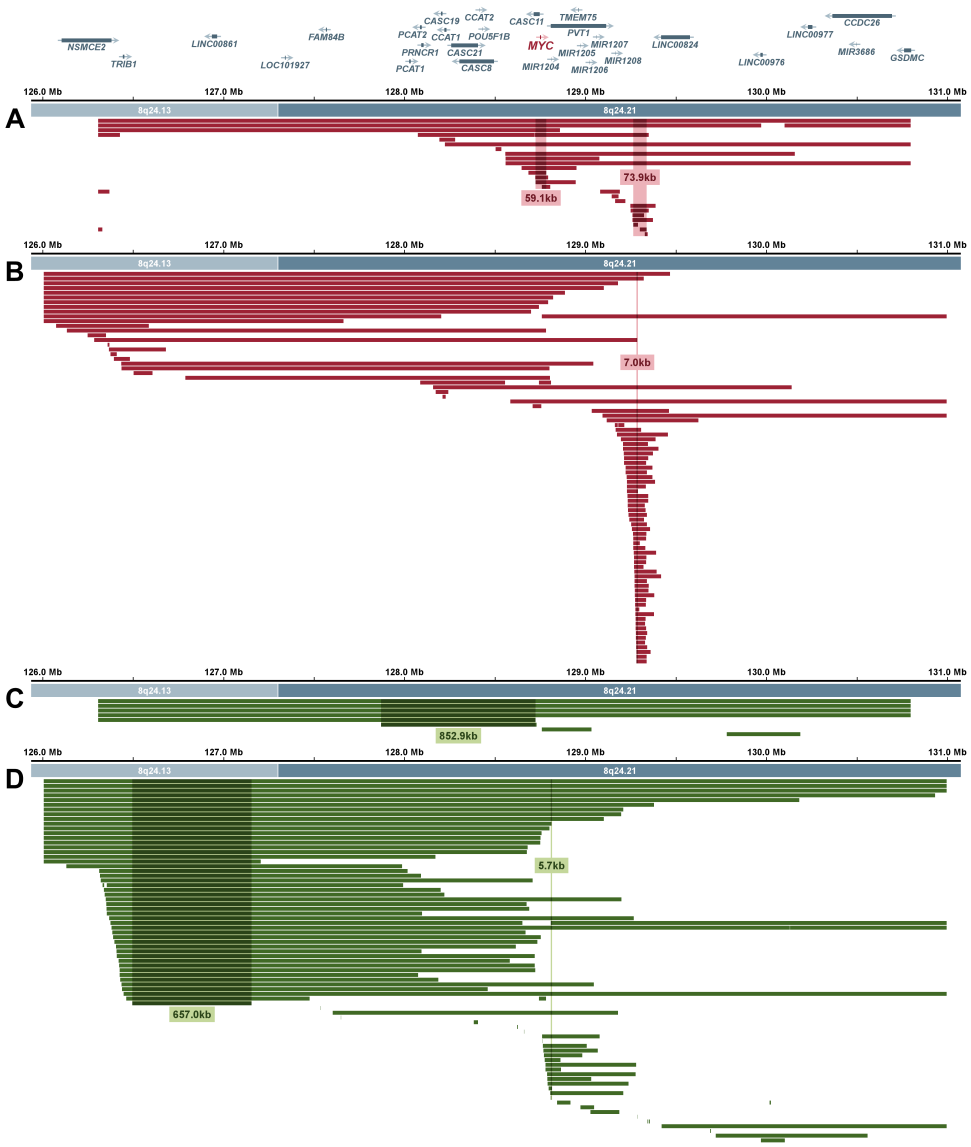
Supplementary Figure 5(D–E): Expression of oncogenes in complex translocations in five cases with available RNA-sequencing data. Box plots show expression distribution of the oncogene in specific *IGH* (left, middle) and *MYC* (right) translocation groups. Red line determines a level of the oncogene expression in the case with complex translocation. Expression was analyzed using RNA-sequencing.



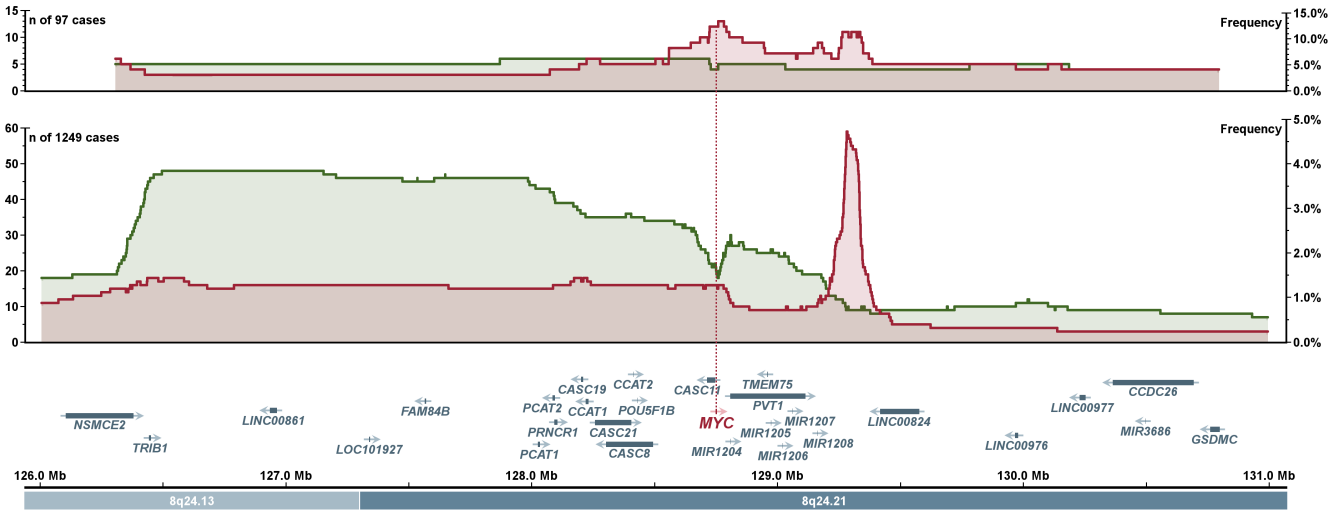
Supplementary Figure 6: Gene-expression microarray analysis of *MYC* in relation to chromosomal abnormalities at 8q24. Effect of abnormality type [**(A)** and **(D)**], translocation category **(B)** and translocation breakpoint position **(C)** are shown. Statistically significant levels are as follows: ***P<0.001, **P<0.01 and *P<0.05.



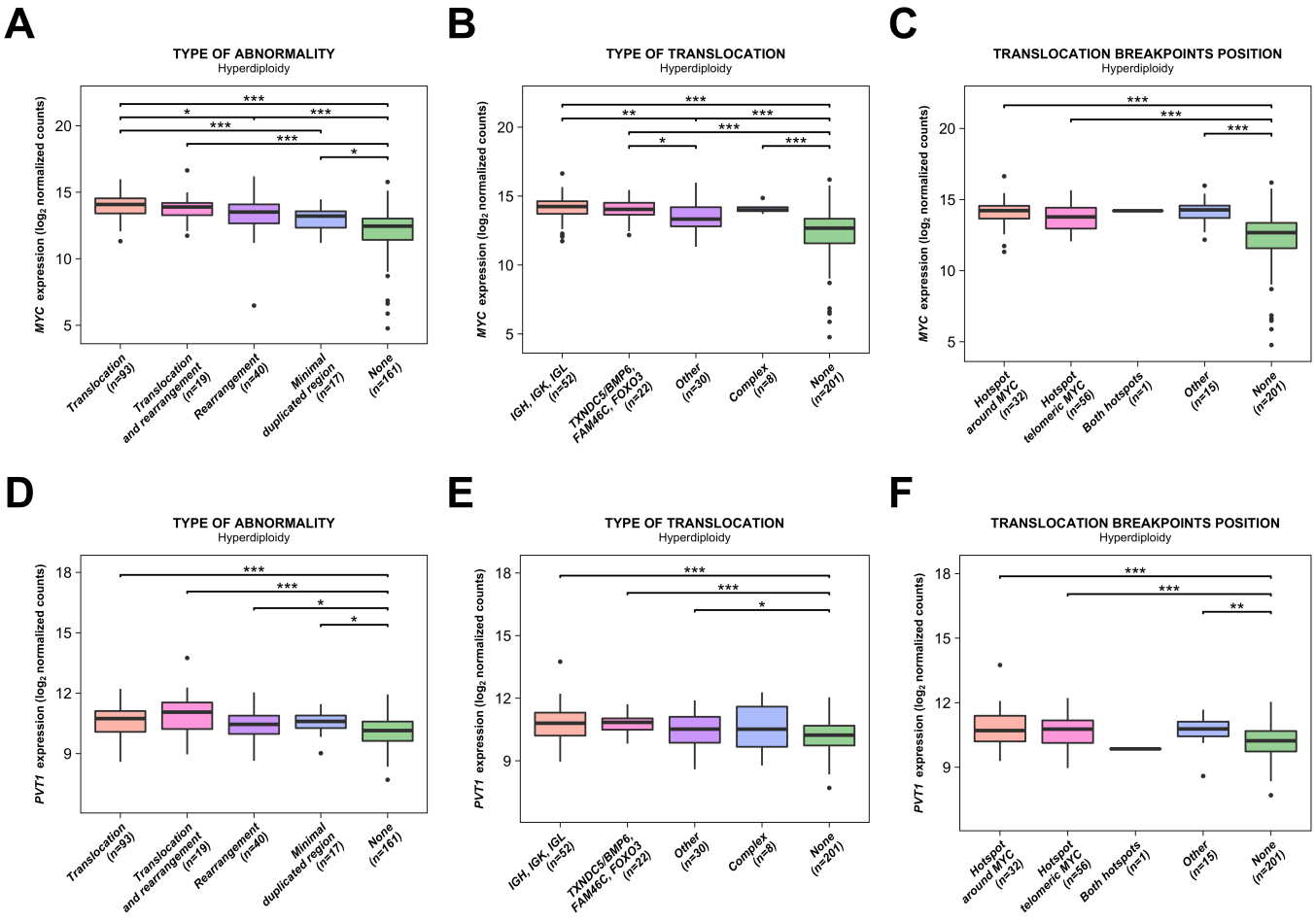
Supplementary Figure 7: Copy-number abnormalities analysis at 8q24. **(A)** Copy-number gains excluding tandem-duplications with two minimal gained regions. **(B)** Tandem-duplications with one minimal tandem-duplicated region. **(C)** Losses excluding deletions with one minimal lost region. **(D)** Deletions with two minimal deleted regions. Tandem-duplication and deletions were tested by paired-end read based analysis in a dataset of 1249 cases. Losses and gains were analyzed using tumor/control ratio depth analysis in a dataset of 97 cases with targeted sequencing. Total of three and 18 cases with complex intra-chromosomal rearrangement (more than five rearrangements) were excluded from analysis. Position of *MYC* (red) and other genes (gray) is shown.



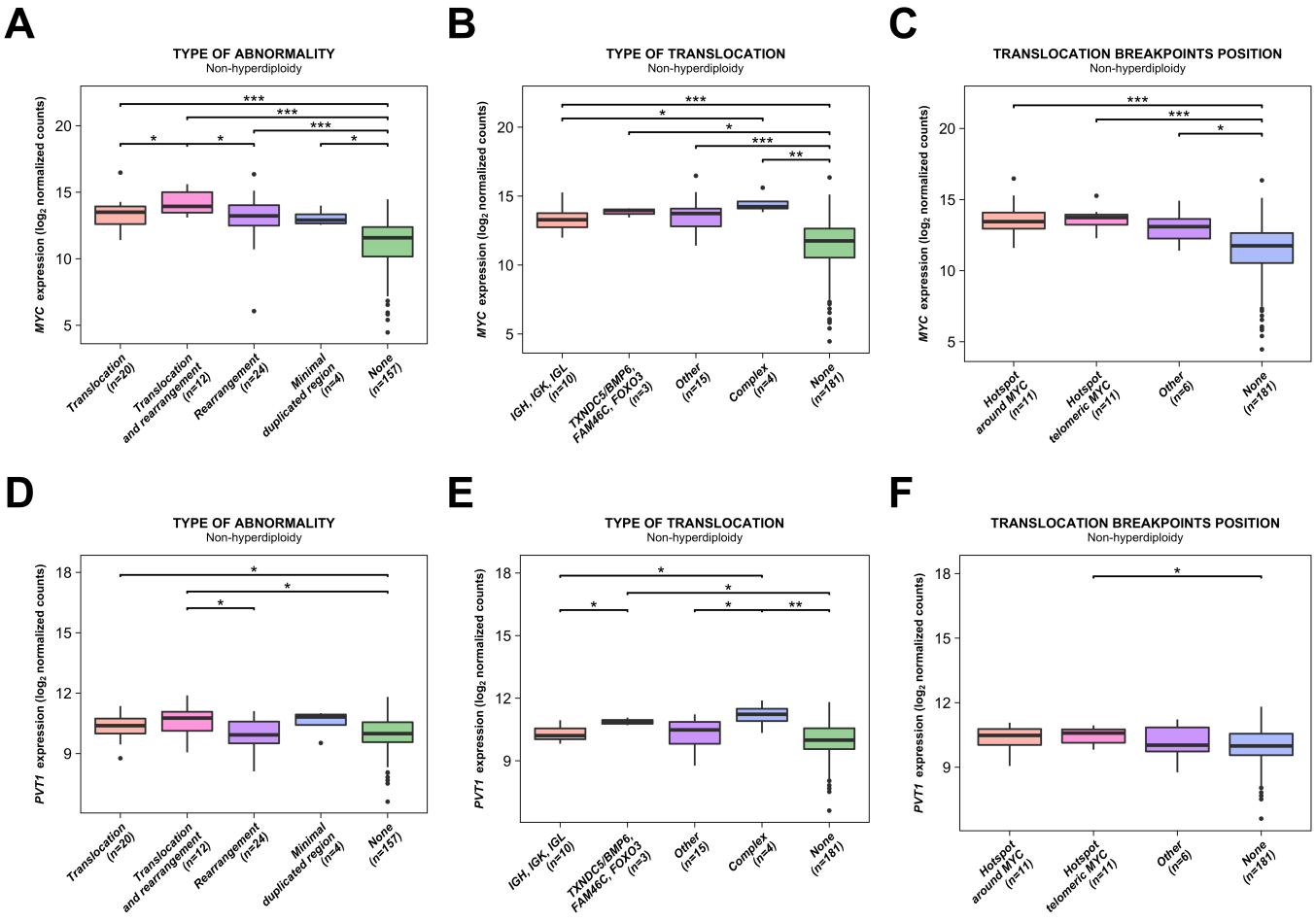
Supplementary Figure 8: Frequency of copy-number abnormalities per position in *MYC* region. Gains (red)/losses (green) are shown in upper part and tandem-duplications (red)/deletions (green) are shown in lower part. Tandem-duplication and deletions were tested by paired-end read based analysis in a dataset of 1249 cases. Losses and gains were analyzed using tumor/control ratio depth analysis in a dataset of 97 cases with targeted sequencing. Total of three and 18 cases with complex intra-chromosomal rearrangement (more than five rearrangements) were excluded from analysis. Position of *MYC* (red) and other genes (gray) is shown.



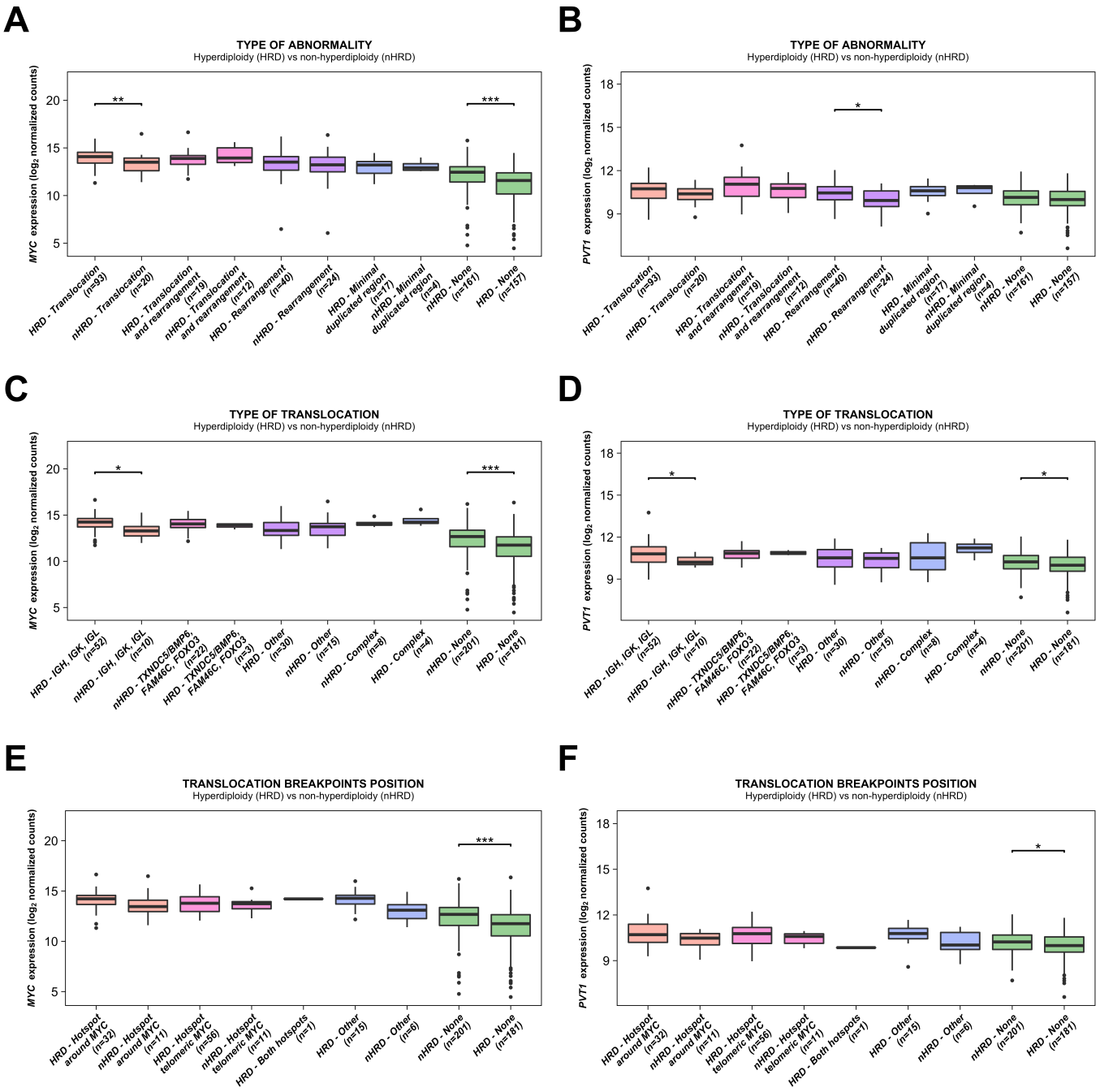
Supplementary Figure 9: RNA-sequencing expression analysis of *MYC* and *PVT1* in relation to chromosomal abnormalities at 8q24 in hyperdiploidy group. Effect of abnormality type [**(A)** and **(D)**], translocation category [**(B)** and **(E)**] and translocation breakpoint position [**(C)** and **(F)**] are shown for *MYC* and *PVT1*, respectively. Statistically significant levels are as follows: ***P<0.001, **P<0.01 and *P<0.05.



Supplementary Figure 10: RNA-sequencing expression analysis of *MYC* and *PVT1* in relation to chromosomal abnormalities at 8q24 in non-hyperdiploidy group. Effect of abnormality type [**(A)** and **(D)**], translocation category [**(B)** and **(E)**] and translocation breakpoint position [**(C)** and **(F)**] are shown for *MYC* and *PVT1*, respectively. Statistically significant levels are as follows: ***P<0.001, **P<0.01 and *P<0.05.



Supplementary Figure 11: RNA-sequencing expression analysis of *MYC* and *PVT1* in relation to chromosomal abnormalities at 8q24 – comparison between hyperdiploidy and non-hyperdiploidy group. Effect of abnormality type [**(A)** and **(D)**], translocation category [**(B)** and **(E)**] and translocation breakpoint position [**(C)** and **(F)**] are shown for *MYC* and *PVT1*, respectively. Statistically significant levels are as follows: ***P<0.001, **P<0.01 and *P<0.05.



Supplementary Alignments

26425_RNAS_D-PL3539_CD138_KP-329MT
t(3;8)

```
chr8:       GGGCACTTCTTGCTTTCTGCCTCCCATCAGTCATCCCAGGGGACGCCAGCTGCCACTTTG
            ||||||||||||||||||||||||||||| ||   |       |   |      ||
KP329:      GGGCACTTCTTGCTTTCTGCCTCCCATCATTCTCTCTGTCTCTCAGAATACTTAACACAT
               | | |     |  |    ||   |  ||||||||||||||||||||||||||||||
chr3:       TTCCTCCTGGAAGTACCCATATCTTTTACTTCTCTCTGTCTCTCAGAATACTTAACACAT
```

37606_RNAS_42485_1-AS-RB-CD138-DNA_CD138_KP-084MT
t(2;8)

```
chr8:       CTGCCAGAGATCCCTGTGTT-AACTGTGAACAGAGCCCTTTTCATCCTCTTGCATCAGAATTCCTG
            |||||||||||||||||||| ||  |    | |       ||||      |||       |  |||
KP084:      CTGCCAGAGATCCCTGTGTT-AATAGACTCCTGCCTGAACTTCAAGGCTATGCCCTGATGTCGCTG
            ||   | |||  |  ||||| ||||||||||||||||||||||||||||||||||||||||||
chr2:       CTTAGAAAGAATCAAGTGTTGAATAGACTCCTGCCTGAACTTCAAGGCTATGCCCTGATGTCGCTG
```

38738_RNAS_51065_1-AS-RB-CD138-DNA_CD138_KP-088MT
t(2;8)

```
chr2:       CTGCTAGAGAGAGTTATGATCTCGCCACTGCACTCCACCCTGTGTGACAGAGTGAGACTC
            ||||||||||||||||||           | | ||    || |        || | |
KP088:      CTGCTAGAGAGAGTTATGATGAGTGGGACCAAGTGCAATAGGTCTATGTCCAGGATAATT
            |            |   ||||||||||||||||||||||||||||||||||||||||||
chr8:       CATCCTTGACTCATCCAGATGAGTGGGACCAAGTGCAATAGGTCTATGTCCAGGATAATT
```

24852_RNAS_D-PL3391_CD138_KP-214MT
t(3;8)

```
chr3:       AAGTCTGATGTGATACCTACAAATTCAGCATATAAAATGAATCTAAAGAGTGCTTTGCT
            |||||||||||||||||||||||||||||||||||    |   |  | |     |
KP214:      AAGTCTGATGTGATACCTACAAATTCAGCATATAAAGGACAGGCATTGGGGTTGCTTTG
              ||  |       |  |     ||  || ||||||||||||||||||||||||||||
chr8:       TCGTTCGTAAACTTCACAGTTTATGAAGTATATAAAGGACAGGCATTGGGGTTGCTTTG
```

27791_RNAS_D-PL3662_CD138_KP-141MT
t(8;19)

```
chr19:      AATCACAGGCACATGCCATCATGCCTGGCTCTTTTTTTT
            |||||||||||| |     |   |     |||
KP141:      AATCACAGGCACAGGAATGAAATTCATTTACTTAAAAAG
            ||  |     ||||||||||||||||||||||||||||
chr8:       AAGAATGACTACAGGAATGAAATTCATTTACTTAAAAAG
```

35250_RNAS_D-PL4968_21925_1-AS-RB-CD138-DNA_CD138_KP-232MT
t(6;8)

```
Chr8:       AGATTATAACCTTTTTAGGAGCAGCACACATTGTACTTACTATATCTTTTTATC
            |||||||||||||||||||||||||||||   |     ||        ||||
KP232:      AGATTATAACCTTTTTAGGAGCAGCACACAGGTTCTCACCTTCTGTGGCTTATT
            |    | |||                 ||||||||||||||||||||||||||||
Chr6:       AAGAGACAACTGACAACCCGAACTTCCACAGGTTCTCACCTTCTGTGGCTTATT
```

39017_RNAS_55603_1-AS-RB-CD138-DNA_CD138_KP-305MT
t(6;8)

```
Chr8:       GTGGGTCTTCATGATGTTCTTGTGATAGTGAGTAAGTCTC
            |||||||||||| |    ||     |       |
KP305:      GTGGGTCTTCATCAGCTCCTCTCTTTCTCTCCTGCACAAG
            |    | ||| |||||||||||||||||||||||||||||
Chr6:       GGCTTTGTTCTTCAGCTCCTCTCTTTCTCTCCTGCACAAG
```

35830_RNAS_29095_1-AS-RB-CD138-DNA_CD138_KP-157MT
t(6;8)

```
Chr8:       GACTAATACTCTTTTACCTAATCAGAGCCTGGCATGGTGCAGGTATATGAAATGA
            |||||||||||||||||||||||||||||||  | ||    |
KP157:      GACTAATACTCTTTTACCTAATCAGAGCCTGGCCGGCTGATTCTCGGGTTGTGCC
              |  |  |     |          ||    ||||||||||||||||||||||
Chr6:       CCCCCAGCCCCAGCTCTGGCCCTGCAGAAATGCCGGCTGATTCTCGGGTTGTGCC
```

Supplementary Tables

Supplementary Table 1: Patients datasets characteristics, techniques for analysis and available number of samples.

	Overall (n=1280)	UAMS (n=100)	UK (n=461)	MMRF (n=706)
		Data availability and method		
Structural changes*	n=1267/1267 (100%)	TS n=100/100 (100%)	WES*** n=461/461 (100%)	WGS n=706/706 (100%)
NS-SNVs	n=1264/1267 (99.8%)	TS n=100/100 (100%)	WES*** n=461/461 (100%)	WES n=703/706 (99.6%)
CNAs**	n=100/1267 (7.9%)	TS n=100/100 (100%)	NA	NA
Gene expression	n=669/1267 (52.8%)	Microarray n=98/100(98.0%)	NA	RNA-Seq n=571/706 (80.9%)
Metadata	n=1262/1267 (99.6%)	n=98/100 (98.0%)	n=461/461 (100%)	n=703/706 (99.6%)
		Basic characteristics		
Median Age [years] (range)	65.0 (30.4-93.0)	60.7 (30.4-75.2)	68.0 (31.0-89.0)	64.0 (31.0-93.0)
Age >= 65 years	672/1262 (53.2%)	27/98 (27.6%)	299/461 (64.9%)	346/703 (49.2%)
ISS stage 1	370/1214 (30.5%)	28/98 (28.6%)	105/436 (24.1%)	237/680 (34.9%)
ISS stage 2	456/1214 (37.6%)	41/98 (41.8%)	169/436 (38.8%)	246/680 (36.2%)
ISS stage 3	388/1214 (32.0%)	29/98 (29.6%)	162/436 (37.2%)	197/680 (29.0%)
t(4;14)	156/1262 (12.4%)	10/98 (10.2%)	58/461 (12.6%)	88/703 (12.5%)
t(6;14)	19/1262 (1.5%)	6/98 (6.1%)	5/461 (1.1%)	8/703 (1.1%)
t(8;14)	7/1164 (0.6%)	ND	1/461 (0.2%)	6/703 (0.9%)
t(11;14)	237/1262 (18.8%)	12/98 (12.2%)	87/461 (18.9%)	138/703 (19.6%)
t(14;16) or t(14;20)	65/1262 (5.2%)	6/98 (6.1%)	20/461 (4.3%)	39/703 (5.5%)

*Translocations and chromosomal rearrangements including deletions, inversions and tandem-duplications

**Copy-number abnormalities analyzed by tumor/control depth ratio

**With custom enrichment for *MYC* region

Supplementary Table 2: List of *MYC* non-synonymous variant in a dataset of 1264 myeloma patients.

n	Protein level	cDNA level	Type of variant	PROVEAN/SIFT prediction
1	p.Ser6Arg	c.18C>G	Missense mutation	Neutral/Damaging
1	p.Pro43fs	c.124delC	Frame-shift deletion	NA/NA
1	p.Ala44Val	c.131C>T	Missense mutation	Neutral/Damaging
1	p.Pro60Ser	c.178C>T	Missense mutation	Deleterious/Damaging
1	p.Val77fs	c.229dupG	Frame-shift insertion	NA/NA
2	p.Ser146Leu	c.437C>T	Missense mutation	Deleterious/Damaging
1	p.Val280del	c.834_836delTGT	In-frame deletion	Deleterious/NA
1	p.Ser420Tyr	c.1259C>A	Missense mutation	Deleterious/Damaging

Supplementary Table 3: Frequency of *MYC* translocation in datasets of 100 patients with targeted sequencing (TS), 461 patients with whole exome sequencing (WES) and 706 patients with whole genome sequencing (WGS).

Dataset	Translocation	Intra-locus rearrangement	Translocation and/or intra-locus rearrangement
TS	29.0% (29/100)	23.0% (23/100)	41.0% (41/100)
WES	23.6% (109/461)	12.8% (59/461)	32.8% (151/461)
WGS	25.6% (181/706)	16.4% (116/706)	37.4% (264/706)
COMBINED	**25.2% (319/1267)**	**15.6% (198/1267)**	**36.0% (456/1267)**

Supplementary Table 4: Proportion of number of chromosomes involved in *MYC* translocation in datasets of 100 patients with targeted sequencing (TS), 461 patients with whole exome sequencing (WES) and 706 patients with whole genome sequencing (WGS).

Dataset	n=2	n=3	n=4	n=5	n>5
TS	69.0% (20/29)	24.1% (7/29)	6.9% (2/29)	0.0% (0/29)	0.0% (0/29)
WES	76.1% (83/109)	18.3% (20/109)	5.5% (6/109)	0.0% (0/109)	0.0% (0/109)
WGS	52.5% (95/181)	25.4% (46/181)	9.9% (18/181)	4.4% (8/181)	7.7% (14/181)
COMBINED	**62.1% (198/319)**	**22.9% (73/319)**	**8.2% (26/319)**	**2.5% (8/319)**	**4.4% (14/319)**

Supplementary Table 5: List of *MYC* translocation partners present in at least five cases in the dataset of 1253 non-complex NDMM patients.

Chromosome band	Position	Size, Mb	Frequency	Super-enhancer--associated genes in MM.1S cell line[1]	Immunoglobulin gene locus	Overlapped high expressed genes*	Candidate genes involved in *MYC* deregulation
14q32.33	chr14:105013903-107220085	2.2	5.0% (63/1253)	*MYC†, TMEM121*	*IGH*	*SIVA1, AKT1, MTA1, IGHG2, IGHA1, IGHG1*	***IGH***
22q11.22/22q11.23	chr22:22658283-24193029	1.5	5.0% (63/1253)	*IGLL5, DERL3, LOC284889, MIF, MIR650, SLC2A11*	*IGL*	*IGLL5, IGLC1, IGLC2, BCR, SMARCB1, DERL3*	***IGL***
6p24.3	chr6:7727323-8387494	0.7	2.7% (34/1253)	*BMP6, MUTED-TXNDC5, TXNDC5, EEF1E1-MUTED, PIP5K1P1*	-	*BMP6, TXNDC5*	***BMP6 TXNDC5***
2p11.2	chr2:88858600-90253854	1.4	2.1% (26/1253)	-	*IGK*	*EIF2AK3, ANKRD36BP2, IGKC*	***IGK***
1p12	chr1:118158927-118431479	0.3	1.6% (20/1253)	*FAM46C*	-	*FAM46C*	***FAM46C***
6q21	chr6:108876006-109352787	0.5	1.1% (14/1253)	*FOXO3*	-	*FOXO3*	***FOXO3***
11q13.4	chr11:72732494-73092358	0.4	0.7% (9/1253)	-	-	*FCHSD2*	***FCHSD2***
11q13.3	chr11:68923361-69978263	1.1	0.6% (8/1253)	-	*IGH* associated	*CCND1‡*	***IGH§***
2p14	chr2:64365459-66730504	2.4	0.5% (6/1253)	*SERTAD2, LOC339807*	-	*PELI1, AFTPH, SERTAD2, SLC1A4, RAB1A, ACTR2*	***SERTAD2***
8q23.3	chr8:113454929-115844684	2.4	0.5% (6/1253)	-	-	-	**unknown**
4q31.3	chr4:153354954-153619440	0.3	0.4% (5/1253)	-	-	*FBXW7*	***FBXW7***
13q22.3	chr13:78500741-78766726	0.3	0.4% (5/1253)	-	-	*MYCBP2* (in <1Mb distance)	***MYCBP2***

* >95% of 571 patients tested by RNA-seq show $\log_2$ normalized counts >10; † Due to the translocation t(8;14) in MM.1S; ‡ In subgroup of patients with t(11;14); § All 8 patients show t(11;14); ‖ Loven *et al.* 2013.

References:

1. Loven J, Hoke HA, Lin CY, *et al.* Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell. 2013;153(2):320-334.

Supplementary Table 6: List of *MYC* translocation partners. n = number of cases in the dataset of 1253 non-complex patients.

Chromosomal band	Genome position	Size, bp	n
1p35.3/1p36.11	chr1:27739863-28392188	652325	2
1p34.3	chr1:35894394-35894431	37	1
1p34.2	chr1:40488361-40756958	268597	2
1p32.3	chr1:52981834	---	1
1p31.3	chr1:66791014-66800178	9164	1
1p22.3	chr1:85996952	---	1
1p22.2	chr1:88948039	---	1
1p12	chr1:118158927-118431479	272552	20
1q21.3	chr1:150651915	---	1
1q23.3	chr1:161726404-163255527	1529123	3
1q25.2	chr1:178531670-178583707	52037	1
1q25.3	chr1:184720646	---	1
1q32.1	chr1:203051534-203274522	222988	2
2p23.3	chr2:25549035-27405899	1856864	2
2p16.2	chr2:54318733-54778431	459698	2
2p14	chr2:64365459-66730504	2365045	6
2p13.3	chr2:70401712	---	1
2p11.2	chr2:88858600-90253854	1395254	26
2q21.2	chr2:134989575	---	1
2q24.3	chr2:166415936	---	1
2q31.1	chr2:173424877-173425255	378	1
2q32.1/2q32.2	chr2:188778708-189976753	1198045	2
3p22.3	chr3:32207945-32208479	534	1
3p21.31	chr3:46330235-46395429	65194	1
3p21.31	chr3:50196574-50389775	193201	1
3p21.1	chr3:53079658	---	1
3q13.2	chr3:112244683-112249926	5243	1
3q26.2	chr3:169228942-169495458	266516	1
3q26.31	chr3:171762090	---	1
4p16.3	chr4:1857489-2732041	874552	2
4p15.2	chr4:25908245-25908304	59	1
4q31.21	chr4:141697852	---	1
4q31.3	chr4:153354954-153619440	264486	5
4q34.3	chr4:179807156	---	1
4q35.1	chr4:185454754-185622688	167934	1

5p14.3/5p15.1	chr5:17929865-19465553	1535688	1
5q11.2	chr5:55401472	---	1
5q14.3	chr5:88450919-88796074	345155	3
5q22.1	chr5:109858587-109859680	1093	1
5q31.2	chr5:139433830	---	1
5q33.1	chr5:149829862-151044833	1214971	2
5q33.3	chr5:156273797-156421100	147303	4
5q34	chr5:160122942-160200603	77661	1
5q35.2	chr5:173129032-173289854	160822	1
6p25.3	chr6:194842-391218	196376	3
6p24.3	chr6:7727323-8387494	660171	34
6p22.3	chr6:21786639-23029400	1242761	1
6p21.2	chr6:37035844-37546594	510750	2
6p21.1	chr6:41858886-41993130	134244	1
6p12.1	chr6:53795877-53961228	165351	1
6q15	chr6:88632133-89925597	1293464	2
6q21	chr6:106041941-107122607	1080666	4
6q21	chr6:108876006-109352787	476781	14
6q22.31	chr6:119693342	---	1
7p21.3	chr7:7914145-7999977	85832	1
7p21.3	chr7:11159783-11593112	433329	1
7p21.2	chr7:13962197-13967016	4819	1
7p15.2	chr7:26008444-26149402	140958	2
7p14.3	chr7:34559624	---	1
7q21.12	chr7:87048847-87049154	307	1
7q22.3	chr7:105450486-105475544	25058	1
7q31.33/7q32.1	chr7:126925518-127156534	231016	1
7q33	chr7:137678731-137683581	4850	1
7q34	chr7:139455673-139639290	183617	2
8p12	chr8:29407990	---	1
8q21.11	chr8:77422720	---	1
8q21.13	chr8:83875044	---	1
8q21.3	chr8:87616372	---	1
8q21.3	chr8:90568607	---	1
8q22.1	chr8:95826016	---	1
8q22.1	chr8:98499039-98652529	153490	3
8q22.2	chr8:101487464	---	1
8q22.3	chr8:103285796-105762724	2476928	3
8q23.1	chr8:106251133	---	1

8q23.2	chr8:111993713	---	1
8q23.3	chr8:113454929-115844684	2389755	6
8q24.12	chr8:119641772-120883792	1242020	2
8q24.22	chr8:132435708-132587826	152118	1
8q24.3	chr8:141922737	---	1
8q24.3	chr8:145287815	---	1
9q21.13	chr9:79164605-79190341	25736	1
9q22.2	chr9:93446606-93817496	370890	1
9q34.11/9q34.13	chr9:132193351-134167408	1974057	2
10p14	chr10:6742071-6868227	126156	1
10q22.3	chr10:79061510-79615885	554375	1
10q24.32	chr10:104146091-104159683	13592	1
10q25.2	chr10:113570048	---	1
10q26.13	chr10:125148066-125191492	43426	1
11p15.1	chr11:19131381-19132109	728	1
11p12	chr11:36704013-37514028	810015	1
11p11.2	chr11:44998507-45076079	77572	1
11q12.1	chr11:58882384-58896224	13840	1
11q13.2	chr11:66775196-66958643	183447	1
11q13.3	chr11:68923361-69978263	1054902	8
11q13.4	chr11:72732494-73092358	359864	9
11q14.1	chr11:82400131-82859140	459009	1
11q22.1	chr11:98859287	---	1
11q23.3	chr11:118938634-119240262	301628	1
11q24.3	chr11:128243023-128717432	474409	2
11q25	chr11:131995275	---	1
12p13.32	chr12:3835029-4707543	872514	3
12p11.23	chr12:26941957	---	1
12p11.21	chr12:32065050	---	1
12q13.11	chr12:47758587	---	1
12q14.1	chr12:58147672-58175116	27444	1
12q15	chr12:68868290-68889029	20739	1
12q22	chr12:93122961	---	1
12q23.3	chr12:105207790-105282218	74428	1
13q13.3	chr13:35705874-35706246	372	1
13q14.2	chr13:48924610	---	1
13q22.3	chr13:78500741-78766726	265985	5
14q23.3	chr14:65720336-65901445	181109	2
14q24.2	chr14:72818022	---	1

14q32.12	chr14:92883617	---	1
14q32.33	chr14:105013903-107220085	2206182	63
15q11.2	chr15:24141512	---	1
15q13.3	chr15:31676325	---	1
15q22.31	chr15:64018516	---	1
15q24.1/15q24.2	chr15:75096204-75459662	363458	2
15q25.1	chr15:80357809-81585731	1227922	2
16p11.2	chr16:29229191-33437673	4208482	4
16q23.1/16q23.2	chr16:78569426-79239195	669769	4
17p13.2	chr17:3624808-4455123	830315	2
17p13.1	chr17:8214064	---	1
17p11.2	chr17:16730207-20401949	3671742	1
17q12	chr17:32532251-32536335	4084	1
17q21.32	chr17:45205683-45354778	149095	1
17q23.3	chr17:62391611-62491820	100209	2
17q25.2	chr17:75110491-75118485	7994	1
18q21.33	chr18:60774868-60841160	66292	1
19p13.3	chr19:1559331-2555073	995742	4
19p13.2	chr19:12804957-13638653	833696	1
19p13.11/19p13.12	chr19:16247562-16599708	352146	2
19q13.32	chr19:46136027	---	1
19q13.33	chr19:48289453-49745349	1455896	1
20p12.2	chr20:9882367-10263026	380659	1
20p12.1	chr20:17819354	---	1
20q11.21	chr20:30814222-30911114	96892	1
20q11.22	chr20:32435299-32623087	187788	3
20q11.22	chr20:34171969-34271613	99644	1
20q13.12/20q13.13	chr20:45975160-47499328	1524168	4
20q13.13	chr20:49076881-49165445	88564	1
21q11.2/21q21.1	chr21:15408264-16910043	1501779	1
21q22.3	chr21:44751330-44832028	80698	1
22q11.22/22q11.23	chr22:22658283-24193029	1534746	63
22q12.1	chr22:28457893-29210349	752456	2
22q13.1/22q13.2	chr22:40604732-42203185	1598453	3
Xq28	chrX:153123577-153313715	190138	1
Un_gl000220unk	chrUn_gl000220:115792-145495	29703	3

Supplementary Table 7: Genes deregulated with *MYC* abnormalities.

Gene symbol	Location	Full name	Regulation*	GEN foldchg	GEN FDR	EPX foldchg	EPX FDR	MYC motif	Evidence
UP-REGULATED									
MYC	8q24.21	*MYC* proto-oncogene, bHLH transcription factor	GEN/EXP	3.5	7.1E-28	5.9	1.6E-67		Up-regulation confirmed in most of the studies
HK2	2p12	hexokinase 2	GEN/EXP	3.6	1.7E-18	2.4	2.3E-08		Validated *MYC* target genes.[3] Up-regulated genes selected in supervised analyses to discriminate cells expressing *MYC* from control cells expressing GFP.[4] Genes up-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC* and down-regulated by the combination of *MYC* and serum.[5]
LAMP5	20p12.2	lysosomal associated membrane protein family member 5	GEN/EXP	3.4	4.0E-07	2.2	2.4E-03		
CGREF1	2p23.3	cell growth regulator with EF-hand domain 1	GEN/EXP	2.9	1.4E-10	2.7	3.6E-09	YES[1,2]	Genes up-regulated in primary epithelial breast cancer cell culture over-expressing *MYC* gene.[4]
DDN	12q13.12	dendrin	GEN/EXP	2.5	1.6E-26	2.5	5.4E-26		
SNHG4	5q31.2	small nucleolar RNA host gene 4	GEN/EXP	2.5	5.1E-24	2.4	2.7E-22		
SORD	15q21.1	sorbitol dehydrogenase	GEN/EXP	2.3	1.6E-26	2.2	2.2E-25	YES[2]	Validated *MYC* target genes.[3] Up-regulated genes selected in supervised analyses to discriminate cells expressing *MYC* from control cells expressing GFP.[4] Genes up-regulated in primary epithelial breast cancer cell culture over-expressing *MYC* gene.[4] Genes up-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) induced to express *MYC*.[6] Genes up-regulated in K562 cells (lymphoblast, chronic myelogenous leukemia) by *MYC* in the presence of *CKN1B*.[7] Genes directly up-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
STEAP3	2q14.2	STEAP3 metalloreductase	GEN/EXP	2.1	1.3E-10	2.3	2.9E-13		
SPTBN2	11q13.2	spectrin beta, non-erythrocytic 2	GEN/EXP	2.3	2.7E-14	2.1	8.4E-12		
VPS9D1-AS1	16q24.3	VPS9D1 antisense RNA 1	GEN/EXP	2.0	5.1E-24	2.2	4.0E-29		
HPDL	1p34.1	4-hydroxyphenylpyruvate dioxygenase like	GEN/EXP	2.1	1.4E-15	2.0	1.0E-13		
RPH3A	12q24.13	rabphilin 3A	GEN/EXP	2.3	5.2E-07	1.8	1.3E-03		
ANKRD13B	17q11.2	ankyrin repeat domain 13B	GEN/EXP	2.0	2.3E-22	2.0	2.9E-24	YES[2]	
SLC19A1	21q22.3	solute carrier family 19 member 1	GEN/EXP	2.0	1.8E-23	1.9	8.3E-20		Validated *MYC* target genes.[3] Up-regulated genes selected in supervised analyses to discriminate cells expressing *MYC* from control cells expressing GFP.[4] Genes up-regulated in primary epithelial breast cancer cell culture over-expressing *MYC* gene.[4] Targets of *MYC* identified by ChIP on chip in cultured cell lines, focusing on E-box-containing genes; high affinity bound subset.[9] Genes identified by ChIP within the high-affinity group of *MYC* targets.[10] Genes whose promoters are bound by *MYC*, according to *MYC* Target Gene Database.[11] Genes directly up-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
SSTR3	22q13.1	somatostatin receptor 3	GEN/EXP	2.1	2.8E-04	1.9	1.9E-03		
SEPT3	22q13.2	septin 3	GEN/EXP	2.2	1.9E-09	1.8	4.9E-05	YES[1,2]	
EPHB4	7q22.1	EPH receptor B4	GEN/EXP	1.9	1.6E-23	1.9	1.3E-19		
MTHFD1L	6q25.1	methylenetetrahydrofolate dehydrogenase	GEN/EXP	1.8	9.1E-11	2.0	6.4E-14		Genes directly up-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]

MFNG	22q13.1	MFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	GEN/EXP	2.0	1.2E-08	1.8	3.0E-06		Targets of *MYC* and *MAX* identified by ChIP on chip in a Burkitt's lymphoma cell line; overlap set.[9] Genes whose promoters are bound by *MYC*, according to *MYC* Target Gene Database.[11] Genes directly up-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
TMEM145	19q13.2	transmembrane protein 145	GEN/EXP	1.8	1.4E-13	2.0	1.2E-18		
HMCN2	9q34.11	hemicentin 2	GEN/EXP	2.0	3.1E-05	1.7	2.7E-03		
LRFN4	11q13.2	leucine rich repeat and fibronectin type III domain containing 4	GEN/EXP	1.8	2.6E-08	1.9	1.2E-10	YES[2]	
GAS5	1q25.1	growth arrest specific 5 (non-protein coding)	GEN/EXP	1.8	1.4E-18	1.9	1.6E-23		
CCDC78	16p13.3	coiled-coil domain containing 78	GEN/EXP	1.8	5.7E-13	1.8	2.2E-12		Up-regulated genes selected in supervised analyses to discriminate cells expressing *MYC* from control cells expressing GFP.[4] Genes up-regulated in primary epithelial breast cancer cell culture over-expressing *MYC* gene.[4]
SVOP	12q24.11	SV2 related protein	GEN/EXP	2.1	2.0E-08	1.6	8.4E-04		
SCN3A	2q24.3	sodium voltage-gated channel alpha subunit 3	GEN	2.1	4.8E-06	1.4	7.7E-02		
ZC3HAV1L	7q34	zinc finger CCCH-type containing, antiviral 1 like	GEN/EXP	1.6	1.7E-05	1.9	4.3E-09		
DIXDC1	11q23.1	DIX domain containing 1	GEN/EXP	1.9	1.2E-09	1.6	4.0E-05		
SLC43A1	11q12.1	solute carrier family 43 member 1	GEN/EXP	1.6	2.3E-05	1.8	2.6E-07	YES[1,2]	Targets of *MYC* and *MAX* identified by ChIP on chip in a Burkitt's lymphoma cell line; overlap set.[9] Genes whose promoters are bound by *MYC*, according to *MYC* Target Gene Database.[11]
S1PR4	19p13.3	sphingosine-1-phosphate receptor 4	GEN/EXP	1.5	4.1E-02	1.9	7.2E-04		
ROR2	9q22.31	receptor tyrosine kinase like orphan receptor 2	GEN/EXP	1.9	1.1E-03	1.5	4.7E-02		Genes down-regulated after double Cre-lox knockout of both *APC* and *MYC* in small intestine.[12] Genes up-regulated after Cre-lox knockout of *APC* in the small intestine that require functional *MYC*.[12] Wnt target genes up-regulated after Cre-lox knockout of *APC* in the small intestine that require functional *MYC*.[12]
SEMA3G	3p21.1	semaphorin 3G	GEN/EXP	1.4	5.6E-03	1.9	6.9E-08		
C4A	6p21.33	complement C4A (Rodgers blood group)	GEN	2.1	1.0E-04	1.2	4.8E-01		
C4A-AS1	6p21.33	C4A antisense RNA 1	GEN	2.0	9.8E-07	1.2	2.8E-01		
C4B-AS1	6p21.33	C4B antisense RNA 1	GEN	2.0	9.8E-07	1.2	2.8E-01		
RELN	7q22.1	reelin	GEN	2.0	3.5E-04	1.2	5.2E-01		
C4B	6p21.33	complement C4B	GEN	2.0	3.0E-04	1.1	7.8E-01		
PTP4A3	8q24.3	protein tyrosine phosphatase type IVA, member 3	GEN	1.9	7.7E-03	1.1	7.1E-01		Genes down-regulated in hepatocellular carcinoma tissue of *MYC* and *TGFA* double transgenic mice.[13]
LDLRAD2	1p36.12	low density lipoprotein receptor class A domain containing 2	GEN	1.8	1.3E-04	1.2	5.1E-01		
DOWN-REGULATED									
MAGED4B	Xp11.22	MAGE family member D4B	GEN/EXP	2.6	1.5E-11	2.4	6.6E-09		
MAGED4	Xp11.22	MAGE family member D4	GEN/EXP	2.6	7.6E-12	2.3	4.0E-09		
CD79A	19q13.2	CD79a molecule	GEN/EXP	2.7	4.0E-08	1.9	7.0E-04		
CD28	2q33.2	CD28 molecule	GEN/EXP	2.1	9.0E-06	2.5	9.4E-08		
PLEKHO1	1q21.2	pleckstrin homology domain containing O1	GEN/EXP	2.8	3.3E-14	1.7	4.3E-04		
NTNG1	1p13.3	netrin G1	GEN/EXP	2.5	7.6E-16	2.0	2.8E-08		
SCNN1B	16p12.2	sodium channel epithelial 1 beta subunit	GEN/EXP	2.4	2.3E-14	2.1	6.5E-10		
CD27	12p13.31	CD27 molecule	GEN/EXP	2.5	9.4E-09	2.0	3.2E-05		Genes down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC* and up-regulated by RNAi knockdown of *TFRC*.[14]

MYADM	19q13.42	myeloid associated differentiation marker	GEN/EXP	1.9	1.0E-03	2.5	5.4E-07	
PTPRCAP	11q13.2	protein tyrosine phosphatase, receptor type C associated protein	GEN/EXP	2.1	2.2E-04	2.3	6.7E-05	
SLC22A17	14q11.2	solute carrier family 22 member 17	GEN/EXP	2.0	4.6E-04	2.4	3.7E-06	
PPIC	5q23.2	peptidylprolyl isomerase C	GEN/EXP	2.0	4.5E-06	2.3	1.0E-07	
LAPTM5	1p35.2	lysosomal protein transmembrane 5	GEN/EXP	2.4	1.0E-05	1.8	8.4E-03	Genes down-regulated in B cell lymphoma tumors expressing an activated form of *MYC*.[15] Genes directly down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
LBH	2p23.1	limb bud and heart development	GEN/EXP	2.2	8.4E-09	2.1	2.5E-07	
RAP1GAP2	17p13.3	RAP1 GTPase activating protein 2	GEN/EXP	2.2	9.0E-13	2.0	4.7E-09	
ARHGEF40	14q11.2	Rho guanine nucleotide exchange factor 40	GEN/EXP	1.9	1.1E-10	2.2	3.0E-15	
TCN2	22q12.2	transcobalamin 2	GEN/EXP	2.0	7.3E-09	2.2	2.7E-11	Genes down-regulated in K562 cells (lymphoblast, chronic myelogenous leukemia) expressing *TP53* and *MYC*.[16] Genes down-regulated by *MYC*, according to the *MYC* Target Gene Database.[11]
BASP1	5p15.1	brain abundant membrane attached signal protein 1	GEN/EXP	1.9	3.3E-04	2.2	1.8E-05	Genes directly down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
PCDHGC3	5q31.3	protocadherin gamma subfamily C,	GEN/EXP	2.4	6.6E-10	1.6	4.3E-03	
CXCL12	10q11.21	C-X-C motif chemokine ligand 12	GEN/EXP	2.0	2.7E-04	2.0	4.7E-04	
MS4A1	11q12.2	membrane spanning 4-domains A1	GEN/EXP	2.0	3.5E-03	2.0	4.4E-03	Genes down-regulated in B cell lymphoma tumors expressing an activated form of *MYC*.[15]
CNN3	1p21.3	calponin 3	GEN/EXP	2.2	3.4E-07	1.7	1.1E-03	
SPRED1	15q14	sprouty related EVH1 domain containing 1	GEN/EXP	2.2	2.5E-10	1.8	1.5E-05	
TMSB4X	Xp22.2	thymosin beta 4, X-linked	GEN/EXP	1.9	3.4E-04	2.0	1.5E-04	Targets of *MYC* and *MAX* identified by ChIP on chip in a Burkitt's lymphoma cell line; overlap set.[9] Genes down-regulated by *MYC*, according to the *MYC* Target Gene Database.[11]
COL9A2	1p34.2	collagen type IX alpha 2 chain	GEN/EXP	2.4	3.4E-09	1.5	3.2E-02	Genes directly down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
AFF2	Xq28	AF4/FMR2 family member	GEN/EXP	2.1	5.1E-06	1.8	1.9E-03	
SGPP1	14q23.2	sphingosine-1-phosphate phosphatase 1	GEN/EXP	1.8	6.1E-08	2.0	5.3E-10	
ADAM28	8p21.2	ADAM metallopeptidase domain 28	GEN/EXP	2.1	1.9E-06	1.7	2.9E-03	
RGS13	1q31.2	regulator of G protein signaling 13	GEN/EXP	1.9	5.0E-03	1.9	3.9E-03	
KIAA0408	6q22.33	KIAA0408	GEN/EXP	2.1	5.8E-14	1.7	8.7E-08	
ZSCAN18	19q13.43	zinc finger and SCAN domain containing 18	GEN/EXP	1.9	1.7E-05	1.9	1.7E-05	
CTHRC1	8q22.3	collagen triple helix repeat containing 1	GEN/EXP	1.9	2.3E-04	1.9	6.0E-04	
MIR155HG	21q21.3	MIR155 host gene	GEN/EXP	2.1	1.5E-04	1.6	3.9E-02	Genes up-regulated by *MYC* and whose promoters are bound by *MYC*, according to *MYC* Target Gene Database.[11] Genes whose promoters are bound by *MYC*, according to *MYC* Target Gene Database.[11]
COL24A1	1p22.3	collagen type XXIV alpha 1 chain	GEN/EXP	1.7	8.7E-07	2.0	2.0E-10	
FGF2	4q28.1	fibroblast growth factor 2	GEN/EXP	2.2	1.0E-08	1.5	1.6E-02	
NRIP1	21q11.2-q21.1	nuclear receptor interacting protein 1	GEN/EXP	1.9	5.6E-10	1.8	5.8E-08	
NR3C2	4q31.23	nuclear receptor subfamily 3 group C member 2	GEN/EXP	2.0	5.0E-14	1.7	6.2E-08	
SV2C	5q13.3	synaptic vesicle glycoprotein 2C	GEN/EXP	1.7	2.5E-04	2.0	4.8E-06	
GBA3	4p15.2	glucosylceramidase beta 3	GEN/EXP	1.9	6.4E-04	1.8	1.5E-03	
OSBPL1A	18q11.2	oxysterol binding protein like 1A	GEN/EXP	2.1	3.0E-09	1.6	5.0E-04	
VPREB3	22q11.23	V-set pre-B cell surrogate light chain 3	GEN	2.1	7.2E-05	1.5	5.2E-02	
SLC40A1	2q32.2	solute carrier family 40 member	GEN/EXP	2.0	4.6E-09	1.6	2.4E-04	

PAX5	9p13.2	paired box 5	GEN/EXP	1.9	4.6E-04	1.7	1.0E-02		Genes directly down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
LINC00494	20q13.13	long intergenic non-protein coding RNA 494	GEN/EXP	1.7	1.1E-06	1.9	2.0E-08		
ATP10B	5q34	ATPase phospholipid transporting 10B (putative)	EXP	1.6	5.1E-02	2.0	1.7E-03		
GNG2	14q22.1	G protein subunit gamma 2	GEN/EXP	1.6	6.9E-04	2.0	1.5E-07		Genes directly down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
RND3	2q23.3	Rho family GTPase 3	GEN	2.2	2.8E-04	1.4	1.8E-01	YES[1]	
TNFSF8	9q32-q33.1	TNF superfamily member 8	GEN/EXP	1.9	1.9E-04	1.7	5.4E-03		
FRMPD3	Xq22.3	FERM and PDZ domain containing 3	GEN/EXP	1.6	1.1E-02	1.9	4.9E-04		
LRP11	6q25.1	LDL receptor related protein 11	GEN/EXP	1.9	1.1E-08	1.6	8.5E-05		
ZCCHC2	18q21.33	zinc finger CCHC-type containing 2	GEN/EXP	1.9	5.6E-07	1.7	1.3E-04		
PTPRJ	11p11.2	protein tyrosine phosphatase, receptor type J	GEN/EXP	1.8	2.1E-06	1.7	1.1E-04		
NEK6	9q33.3	NIMA related kinase 6	GEN/EXP	2.1	3.0E-08	1.5	1.3E-02	YES[2]	
ALOX5AP	13q12.3	arachidonate 5-lipoxygenase activating protein	GEN/EXP	1.7	6.6E-03	1.9	9.4E-04		Genes positively correlated with amplifications of *MYC* in small cell lung cancer cell lines.[17]
DMKN	19q13.12	dermokine	GEN/EXP	1.5	6.1E-03	2.0	2.3E-07		
TIAM1	21q22.11	T-cell lymphoma invasion and metastasis 1	GEN/EXP	1.8	7.9E-06	1.7	2.0E-04		Wnt target genes up-regulated after Cre-lox knockout of *APC* in the small intestine that require functional *MYC*.[12] Genes that interact with *MYC* by Genomatix MatBase database of transcription factors.[18]
SH3TC1	4p16.1	SH3 domain and tetratricopeptide repeats 1	GEN/EXP	1.9	9.0E-08	1.6	3.2E-04		
ZYX	7q34	zyxin	GEN/EXP	1.9	1.1E-07	1.6	1.1E-04		
PARM1	4q13.3	prostate androgen-regulated mucin-like protein 1	GEN/EXP	1.8	2.3E-10	1.6	7.7E-07		
WNT5B	12p13.33	Wnt family member 5B	GEN/EXP	1.7	2.1E-06	1.8	5.5E-08		Genes down-regulated in primary epithelial breast cancer cell culture over-expressing *MYC* gene.[4]
B3GALNT1	3q26.1	beta-1,3-N-acetylgalactosaminyltransferase 1 (globoside blood group)	GEN/EXP	1.8	9.4E-06	1.7	2.9E-04		
PTPRC	1q31.3-q32.	protein tyrosine phosphatase, receptor type C	GEN/EXP	1.9	1.2E-05	1.5	1.0E-02		Genes down-regulated in B cell lymphoma tumors expressing an activated form of *MYC*.[15] Genes down-regulated in K562 cells (lymphoblast, chronic myelogenous leukemia) by *MYC* in the presence of *CKN1B*.[7] Genes directly down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
LPGAT1	1q32.3	lysophosphatidylglycerol acyltransferase 1	GEN/EXP	1.8	2.0E-08	1.6	1.6E-05		
MIAT	22q12.1	myocardial infarction associated transcript (non-protein coding)	GEN/EXP	1.6	2.3E-02	1.8	1.3E-03		
RIMS3	1p34.2	regulating synaptic membrane exocytosis 3	GEN/EXP	1.9	1.9E-10	1.4	2.2E-03		
FRMD6	14q22.1	FERM domain containing 6	GEN/EXP	1.9	1.1E-08	1.4	7.5E-03		
RASGRP3	2p22.3	RAS guanyl releasing protein 3	GEN/EXP	1.9	8.0E-11	1.4	2.7E-03		
CRIM1	2p22.2	cysteine rich transmembrane BMP regulator 1	GEN/EXP	1.4	2.9E-02	1.9	1.1E-05		
SOCS1	16p13.13	suppressor of cytokine signaling 1	GEN/EXP	1.9	4.5E-08	1.4	4.8E-03		Genes directly down-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
NLGN4X	Xp22.32-p22.31	neuroligin 4, X-linked	EXP	1.5	7.3E-02	1.8	6.7E-03		
SLC44A2	19p13.2	solute carrier family 44 member 2	GEN/EXP	1.5	1.6E-03	1.8	3.9E-07		
BMP2K	4q21.21	BMP2 inducible kinase	GEN/EXP	1.9	2.8E-11	1.4	3.2E-03	YES[1,2]	
AHR	7p21.1	aryl hydrocarbon receptor	GEN/EXP	1.8	4.9E-07	1.5	4.3E-03		Genes that regulate *MYC* by Genomatix MatBase database of transcription factors.[18]
DSG2	18q12.1	desmoglein 2	EXP	1.2	4.1E-01	2.0	1.8E-03		Genes directly up-regulated in P493-6 cells (B lymphocyte, Burkitt's lymphoma) by *MYC*.[8]
MYOF	10q23.33	myoferlin	GEN/EXP	1.4	4.7E-02	1.9	1.0E-04		

GRASP	12q13.13	general receptor for phosphoinositides 1 associated scaffold protein	GEN/EXP	1.8	7.3E-05	1.5	2.4E-02		
SYNPO	5q33.1	synaptopodin	GEN/EXP	1.4	4.9E-03	1.8	1.6E-06		
WDFY3	4q21.23	WD repeat and FYVE domain containing 3	GEN/EXP	1.9	2.4E-06	1.4	2.3E-02		
TNFSF12	17p13.1	TNF superfamily member 12	GEN/EXP	1.8	1.1E-05	1.4	3.6E-02		
RRAS2	11p15.2	RAS related 2	GEN	2.0	1.7E-05	1.2	4.3E-01		
DOK4	16q21	docking protein 4	GEN/EXP	1.8	1.1E-11	1.4	2.0E-03		
BIRC3	11q22.2	baculoviral IAP repeat containing 3	GEN	2.1	5.1E-06	1.1	7.7E-01		Genes whose promoters are bound by *MYC*, according to *MYC* Target Gene Database.[11]
KCNN4	19q13.31	potassium calcium-activated channel subfamily N member 4	GEN	1.9	4.1E-04	1.3	3.2E-01	YES[1,2]	Genes up-regulated hT-RPE cells (immortalized retinal pigment epithelium) by *MYC*.[19]

*__GEN/EXP__: gene was significantly de-regulated in cases with abnormal *MYC* genomic profile as well as in cases with *MYC* expression log2>=13.0 with fold-change >=1.8 at least in one of these two tested parameters. __GEN__: gene was significantly de-regulated in cases with abnormal *MYC* genomic profile with fold-change >=1.8 and not significant in cases with *MYC* expression log2>=13.0. __EXP__: gene was significantly de-regulated in cases with *MYC* expression log2>=13.0 with fold-change >1.8 and not significant in cases with abnormal *MYC* genomic profile

References:

1. Wingender E. The TRANSFAC project as an example of framework technology that supports the analysis of genomic regulation. Brief Bioinform. 2008;9(4):326-332.
2. Xie X, Lu J, Kulbokas EJ, et al. Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. Nature. 2005;434(7031):338-345.
3. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1(6):417-425.
4. Bild AH, Yao G, Chang JT, et al. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. Nature. 2006;439(7074):353-357.
5. Schlosser I, Holzel M, Hoffmann R, et al. Dissection of transcriptional programmes in response to serum and c-Myc in a human B-cell line. Oncogene. 2005;24(3):520-524.
6. Schuhmacher M, Kohlhuber F, Holzel M, et al. The transcriptional program of a human B cell line in response to Myc. Nucleic Acids Res. 2001;29(2):397-406.
7. Acosta JC, Ferrandiz N, Bretones G, et al. Myc inhibits p27-induced erythroid differentiation of leukemia cells by repressing erythroid master genes without reversing p27-mediated cell cycle arrest. Mol Cell Biol. 2008;28(24):7286-7295.
8. Zeller KI, Zhao X, Lee CW, et al. Global mapping of c-Myc binding sites and target gene networks in human B cells. Proc Natl Acad Sci U S A. 2006;103(47):17834-17839.
9. Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet. 2008;40(5):499-507.
10. Fernandez PC, Frank SR, Wang L, et al. Genomic targets of the human c-Myc protein. Genes Dev. 2003;17(9):1115-1129.
11. Zeller KI, Jegga AG, Aronow BJ, O'Donnell KA, Dang CV. An integrated database of genes responsive to the Myc oncogenic transcription factor: identification of direct genomic targets. Genome Biol. 2003;4(10):R69.
12. Sansom OJ, Meniel VS, Muncan V, et al. Myc deletion rescues Apc deficiency in the small intestine. Nature. 2007;446(7136):676-679.
13. Lee JS, Chu IS, Mikaelyan A, et al. Application of comparative functional genomics to identify best-fit mouse models to study human cancer. Nat Genet. 2004;36(12):1306-1311.
14. O'Donnell KA, Yu D, Zeller KI, et al. Activation of transferrin receptor 1 by c-Myc enhances cellular proliferation and tumorigenesis. Mol Cell Biol. 2006;26(6):2373-2386.
15. Yu D, Cozma D, Park A, Thomas-Tikhonenko A. Functional validation of genes implicated in lymphomagenesis: an in vivo selection assay using a Myc-induced B-cell tumor. Ann N Y Acad Sci. 2005;1059(145-159.
16. Ceballos E, Munoz-Alonso MJ, Berwanger B, et al. Inhibitory effect of c-Myc on p53-induced apoptosis in leukemia cells. Microarray analysis reveals defective induction of p53 target genes and upregulation of chaperone genes. Oncogene. 2005;24(28):4559-4571.
17. Kim YH, Girard L, Giacomini CP, et al. Combined microarray analysis of small cell lung cancer reveals altered apoptotic balance and distinct expression signatures of MYC family gene amplification. Oncogene. 2006;25(1):130-138.
18. Genomatix Matrix Library v11.0 (www.genomatix.de)
19. Alfano D, Votta G, Schulze A, et al. Modulation of cellular migration and survival by c-Myc through the downregulation of urokinase (uPA) and uPA receptor. Mol Cell Biol. 2010;30(7):1838-1851.