SUPPLEMENTARY METHODS

PATIENT SAMPLES AND DNA ISOLATION

Follow up samples were taken at least 21 days after the start of the last induction cycle. If additional CR samples were available, the sample preceding consolidation therapy was chosen. When patients did not receive any consolidation therapy, the last sample within four months after the start of the last induction cycle was selected.

The majority of AML samples were purified by Ficoll-Hypaque centrifugation (Nygaard, Oslo, Norway), cryopreserved and subsequently lysed in RLT buffer (Qiagen, Venlo, the Netherlands). High quality DNA was extracted using the QIASymphony DSP DNA Mini Kit according to the manufacturer's instructions (Qiagen, Venlo, the Netherlands). DNA concentration was measured by Qubit Fluorometric Quantitation (Thermo Fisher Scientific, Wilmington, DE).

TARGETED NGS AND DATA ANALYSIS

The TruSight Myeloid Sequencing Panel libraries were paired-end sequenced (2x221bp) on an Illumina HiSeq 2500 System (Illumina, San Diego, CA) in Rapid Run mode or on a Illumina MiSeq System (Illumina, San Diego, CA). The vast majority of amplicon target regions were completely paired-end sequenced. Overlap-based error-correction was utilized to attenuate any form of strand-specific error biases. Error-corrected paired-end reads aligned to the human genome version 19 (hg19) with BBMAP3 followed by quality control to determine cases with insufficient number of reads for adequate variant calling. Single nucleotide variants (SNVs) and insertions-deletions (indels) at diagnosis were determined by MuTect4, Samtools5, GATK6, Varscan7, Indelocator8 and Pindel6. Variant allele frequencies (VAF) of mutations detected at diagnosis were calculated as the ratio between the number of mutant and total reads. Pathogenic *TP53* variants were defined by occurrence in the COSMIC and IARC TP53 database as well as by analyses in silico with programs, such as Polyphen-2, SIFT, FATHMM, MetaSVM, MetaLR, CADD, DANN and ClinVar.

TP53 DEEP SEQUENCING

For deep sequencing template specific primers for *TP53* mutation NGS analysis were designed by using the Ion AmpliSeq Designer software (ThermoFisher Scientific, Bleiswijk). The *TP53* specific primers (see below) were adapted for Illumina-based sequencing by adding an Illumina forward (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'-[locus-specific sequence] or reverse (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3')-[locus-specific sequence] overhang adapter sequence to the *TP53* specific primers.

In the second PCR, by means of these adapter sequences, sample-specific dual indices for sample identification and Illumina sequencing adapters are attached by using unique dual index primers from the IDT for Illumina Nextera DNA UD Index Kit (Illumina, San Diego, CA, USA). Two multiplex reactions were carried out (see below). The *TP53* loci of interest were amplified by multiplex PCR on 100ng genomic DNA using the Roche FastStart High Fidelity PCR System (Roche) containing 1× Buffer with 1.8mM MgCl2, 0.2mM dNTP, ~0.4µM each primer, 0.1U FastStart Taq DNA polymerase. Amplification was performed using the following thermocycling conditions: 95°C for 5 minutes, 25 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C and a final extension for 7 minutes at 72°C. Amplicons from the first step PCR were purified using the Agencourt AMPure XP bead purification kit (Beckman Coulter, Fullerton, CA, USA).

The second step PCR was performed with primers from the IDT for Illumina Nextera DNA UD Index Kit (Illumina, San Diego, CA) using the KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA, USA) with the following thermocycling conditions: 95°C for 3 minutes, 10 cycles of 20 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C and a final extension for 5 minute at 72°C.

The library pool was purified with Agencourt AMPure XP beads and normalized for Illumina-based sequencing, according to the manufacturer's protocol (Illumina, San Diego, CA). The NGS libraries were paired-end sequenced (2×221-bp) on the MiSeq System (Illumina, San Diego, CA) according to manufacturer's recommendation (Illumina, San Diego, CA). NGS data analysis to detect variants at a

low detection level is performed as described before (Jongen-Lavrencic et al., 2018). The maximum limit of detection in the follow up samples was VAF 0.001% (dependent on type of *TP53* mutation).

Primer name	Sequence (5' -3')
TP53_AMP1_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCTTCTGACGCACACCTATTG
TP53_AMP1_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCATGTTCAAGACAGAAGGGCCTGA
TP53_AMP2_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAATCCTATGGCTTTCCAACCTAG
TP53_AMP2_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCAGAGCTGAATGAGGCCTTGGAACT
TP53_AMP3_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTcaggctaggctaagctatgatgttccttaga
TP53_AMP3_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCTTGTTAAAGAGAGCATGAAAATGGTTCT
TP53_AMP4_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAAACGGCATTTTGAGTGTTAGACTGGA
TP53_AMP4_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCTTTCCTTGCCTCTTTCCTAGCACTG
TP53_AMP5_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAACTGCACCCTTGGTCTCCTC
TP53_AMP5_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCAACAGCTTTGAGGTGCGTGTTTG
TP53_AMP6_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAATCGGTAAGAGGTGGGCCCAG
TP53_AMP6_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCCACTACAACTACATGTGTAACAGTTCC
TP53_AMP7_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTttcaactgtgcaatagttaaacccat
TP53_AMP7_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCTGAGGTCTGGTTTGCAACTGGG
TP53_AMP8_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATCCAAATACTCCACACGCAAATTTC
TP53_AMP8_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCGCTGCTCAGATAGCGATG
TP53_AMP9_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGCTGTGACTGCTTGTAGATGGC
TP53_AMP9_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCCTGACTTTCAACTCTGTCTCCTTCCTC
TP53_AMP10_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAGCCAAAGGGTGAAGAGGAATCCC
TP53_AMP10_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCAAGTCTGTGACTTGCACGGTCAGTTG
TP53_AMP11_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAGCTGCCCTGGTAGGTTTTC
TP53_AMP11_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCACTGAAGACCCAGGTCCAGAT
TP53_AMP12_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTccccagcccAACCCTTGTCCTT
TP53_AMP12_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCAGCCCCCTAGCAGAGACCTG
TP53_AMP13_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGAACAAGAAGTGGAGAATGTCAG
TP53_AMP13_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCATGTGATGTCATCTCTCCTCCCTGCTT
TP53_AMP14_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTCCCAGCCTGGGCATCCTT
TP53_AMP14_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCaaccatcttttaactcaggtactgtGT
TP53_AMP15_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTatcgtaagtcaagtagcatctgTATCA
TP53_AMP15_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCGCTCCTGGTTGTAGCTAACTAACTTC
TP53_AMP16_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAGGAGCTGGTGTTGTTGGG
TP53_AMP16_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCTAAGCGAGGTAAGCAAGCAGGAC
TP53_AMP17_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTTTCTTGCGGAGATTCTCTTCCTCT
TP53_AMP17_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCGGACAGGTAGGACCTGATTTCCTT
TP53_AMP18_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTCTTCCAGTGTGATGATGGTGAG
TP53_AMP18_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCTTGCCACAGGTCTCCCCAAGG
TP53_AMP19_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCACTGACAACCACCCTTAACCC
TP53_AMP19_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCTCTGGCCCCTCCTCAGCATCT
TP53_AMP20_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCAGCCCCAGCTGCTCAC
TP53_AMP20_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCTTGCCAACTGGCCAAGACCT
TP53_AMP21_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAAACATCTTGTTGAGGGCAGGG
TP53_AMP21_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCCTGAGGTGTAGACGCCAACTCTC
TP53_AMP22_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCATTGAAGTCTCATGGAAGCCA
TP53_AMP22_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCTCCTGGCCCCTGTCATCTTCTGT
TP53_AMP23_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAGCAGCCTCTGGCATTCTG
TP53_AMP23_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCTCTGACTGCTCTTTTCACCCATC
TP53_AMP24_Rd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGCCCTTCCAATGGATCCACTC
TP53_AMP24_Rd2	TCGCGAGTTAATGCAACGATCGTCGAAATTCGCAGGGTTGGAAGTGTCTCATGCTGGA

Primer mix 1	Primerstock (pmol/µl)	Pool primerstock (µl)
TP53_AMP1_Rd1	200	5
TP53_AMP1_Rd2	200	5
TP53_AMP2_Rd1	200	5
TP53_AMP2_Rd2	200	5
TP53_AMP3_Rd1	200	5
TP53_AMP3_Rd2	200	5
TP53_AMP4_Rd1	200	5
TP53_AMP4_Rd2	200	5
TP53_AMP5_Rd1	200	5
TP53_AMP5_Rd2	200	5
TP53_AMP6_Rd1	200	5
TP53_AMP6_Rd2	200	5
TP53_AMP7_Rd1	200	5
TP53_AMP7_Rd2	200	5
TP53_AMP8_Rd1	200	5
TP53_AMP8_Rd2	200	5
TP53_AMP9_Rd1	200	5
TP53_AMP9_Rd2	200	5
TP53_AMP10_Rd1	200	5
TP53_AMP10_Rd2	200	5
TP53_AMP11_Rd1	200	5
TP53_AMP11_Rd2	200	5
TP53_AMP12_Rd1	200	5
TP53_AMP12_Rd2	200	5

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Primer mix 2	Primerstock (pmol/µl)	Pool primerstock (µl)
TP53_AMP13_Rd1	200	5
TP53_AMP13_Rd2	200	5
TP53_AMP14_Rd1	200	5
TP53_AMP14_Rd2	200	5
TP53_AMP15_Rd1	200	20
TP53_AMP15_Rd2	200	20
TP53_AMP16_Rd1	200	5
TP53_AMP16_Rd2	200	5
TP53_AMP17_Rd1	200	5
TP53_AMP17_Rd2	200	5
TP53_AMP18_Rd1	200	2.5
TP53_AMP18_Rd2	200	2.5
TP53_AMP19_Rd1	200	5
TP53_AMP19_Rd2	200	5
TP53_AMP20_Rd1	200	5
TP53_AMP20_Rd2	200	5
TP53_AMP21_Rd1	200	2.5
TP53_AMP21_Rd2	200	2.5
TP53_AMP22_Rd1	200	5
TP53_AMP22_Rd2	200	5
TP53_AMP23_Rd1	200	5
TP53_AMP23_Rd2	200	5
TP53_AMP24_Rd1	200	5
TP53_AMP24_Rd2	200	5

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Table S1.
Molecular characteristics of mutant *TP53* AML/MDS-EB by complex karyotype.

Table S1. Molecular characteristics of mutant *TP53* AML/MDS-EB by complex karyotype.			
	Complex karyotype (n=185)	**Non-complex karyotype (n=35)**	
***TP53* mutant allelic status - no. (%)**			p<0.001
mono-allelic	20 (11)	30 (86)	
bi-allelic	165 (89)	5 (14)	
No. of *TP53* mutations - no. (%)			p=0.044
Single	140 (76)	32 (91)	
Multiple	45 (24)	3 (9)	
***TP53* clone size - VAF (%)**			p<0.001
Median	53	11	
Range	2-97	1-97	
Cytogenetics - no. (%)			
monosomy 5	62 (34)	0 (0)	p<0.001
deletion 5q	101 (55)	3 (9)	p<0.001
monosomy 7	68 (37)	4 (11)	p=0.003
monosomy 17	80 (43)	1 (3)	p<0.001
abnormality 17p	39 (21)	0 (0)	p=0.001
monosomal karyotype	171 (92)	3 (9)	p<0.001
Mutation at diagnosis - no. (%)			
any concurrent	84 (45)	26 (74)	p=0.003
DNMT3A	25 (14)	6 (17)	p=0.597
TET2	16 (9)	4 (11)	p=0.534
ASXL1	6 (3)	5 (14)	p=0.017
RUNX1	6 (3)	5 (14)	p=0.017
SRSF2	4 (2)	7 (20)	p<0.001

Figure S1.
Consort diagram of mutant *TP53* study. Abbreviations: HO, HOVON-SAKK, Dutch-Belgian Hemato-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research.

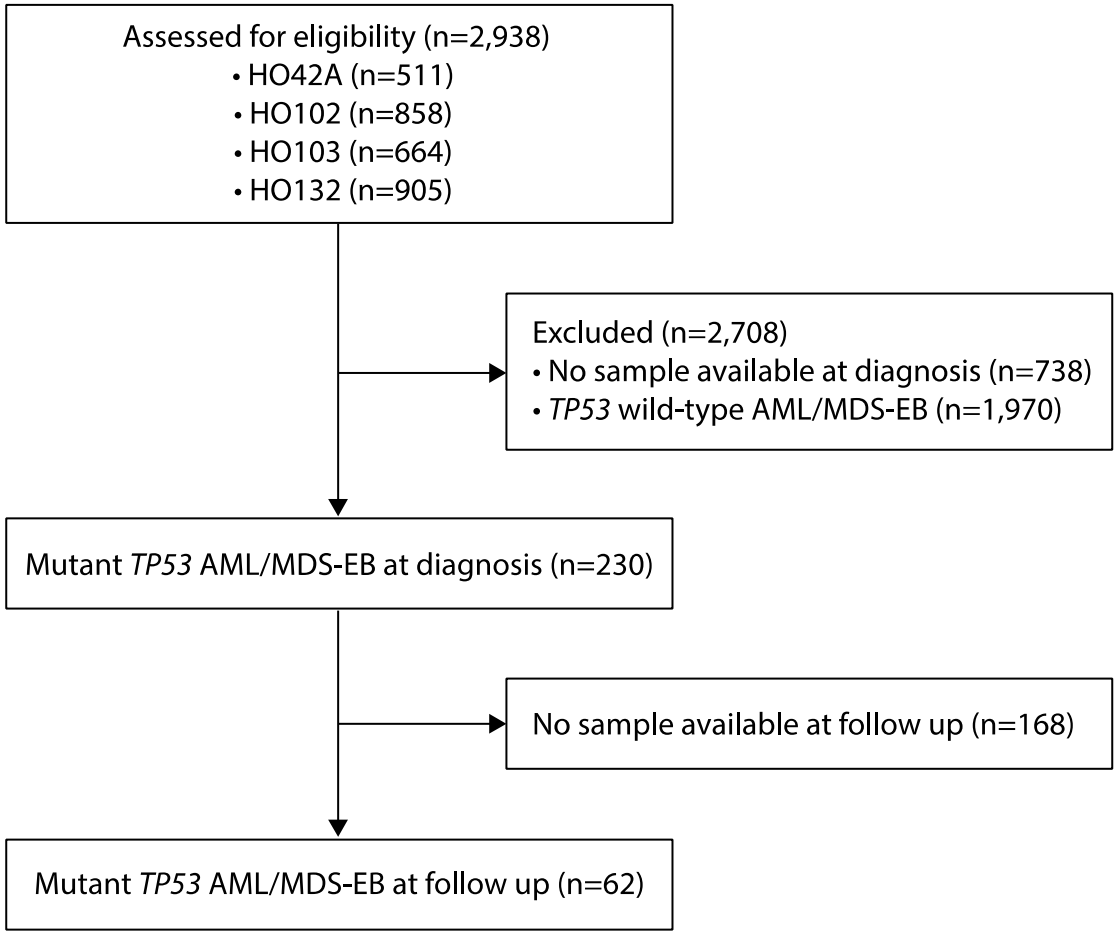


Figure S2.
Consort diagram of the mutant *TP53* allelic status of 230 AML/MDS-EB patients. AML/MDS-EB patients with multipele *TP53* mutations or with mutant *TP53* combined with concurrent chromosome 17 aberrations (eg. monosomy 17 of abnormality 17p) were assigned as bi-allelic *TP53* mutant. In *TP53* AML/MDS-EB without chromosome 17 aberrations, patients with a mutant *TP53* variant allele frequency above 55%, were also considered bi-allelic *TP53* mutant. In 10 patients cytogenetic data was missing. Of note, 4 patients with missing cytogenetics were considered bi-allelic *TP53* mutant based on the presence of multiple *TP53* mutations or a variant allele frequency above 55%.

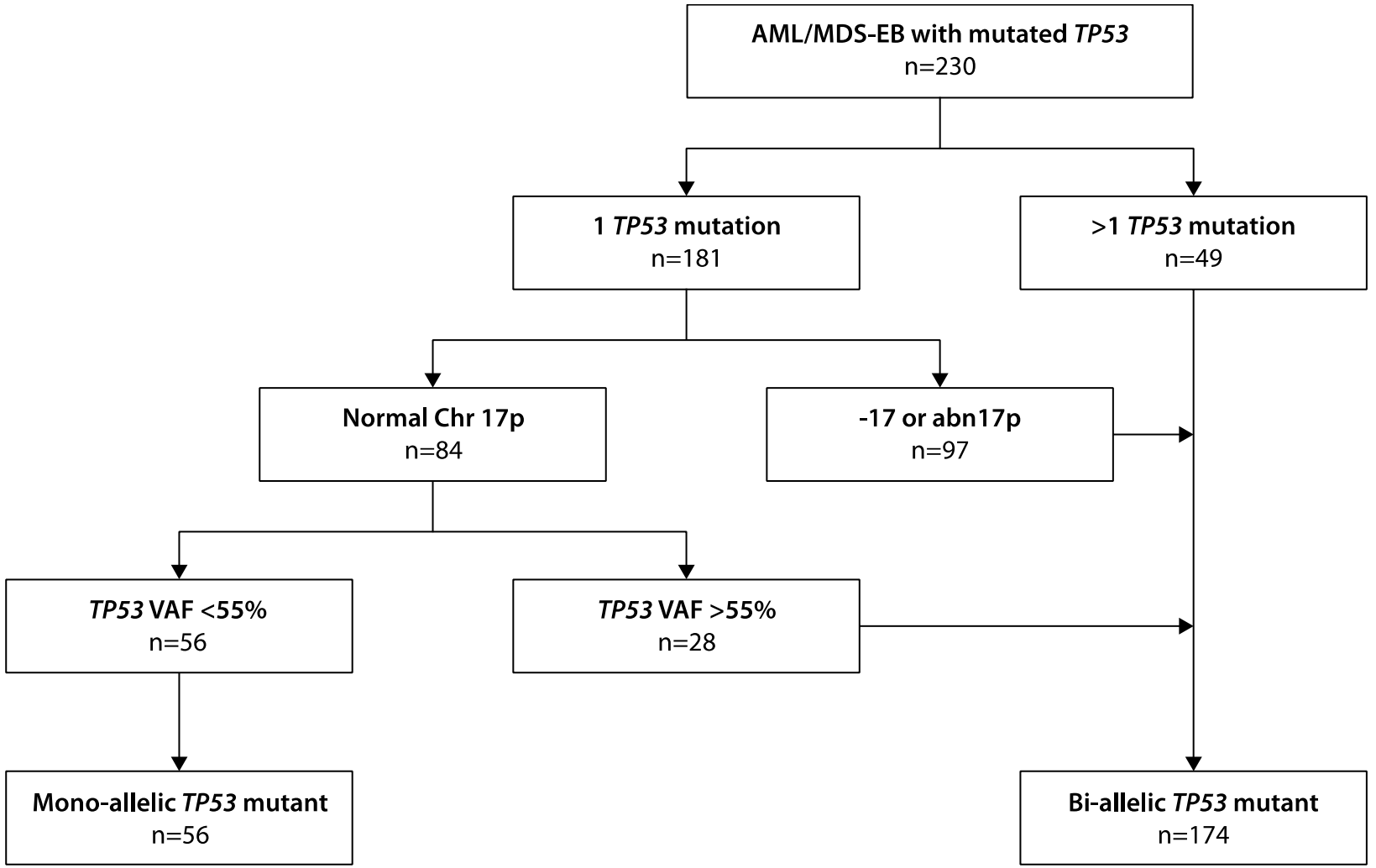


Figure S3.
Lollipop plot illustrating the distribution of 228 *TP53* gene variants in 230 mutant *TP53* AML/MDS-EB patients. The variants shown are depicted by using the NM_000546 transcript reference sequence. Functional protein domains are indicated in purple (TAD: Trans-activation domain), orange (DNA binding domain) and yellow (oligomerization domain). The upper panel shows the distribution of missense mutations (green) and the lower panel the nonsense and indel mutations. The Y-axis represent the number of detected gene variants. Splice-site mutations (n=23) are not reported and 32 gene variants are out of scope of the reference sequence.

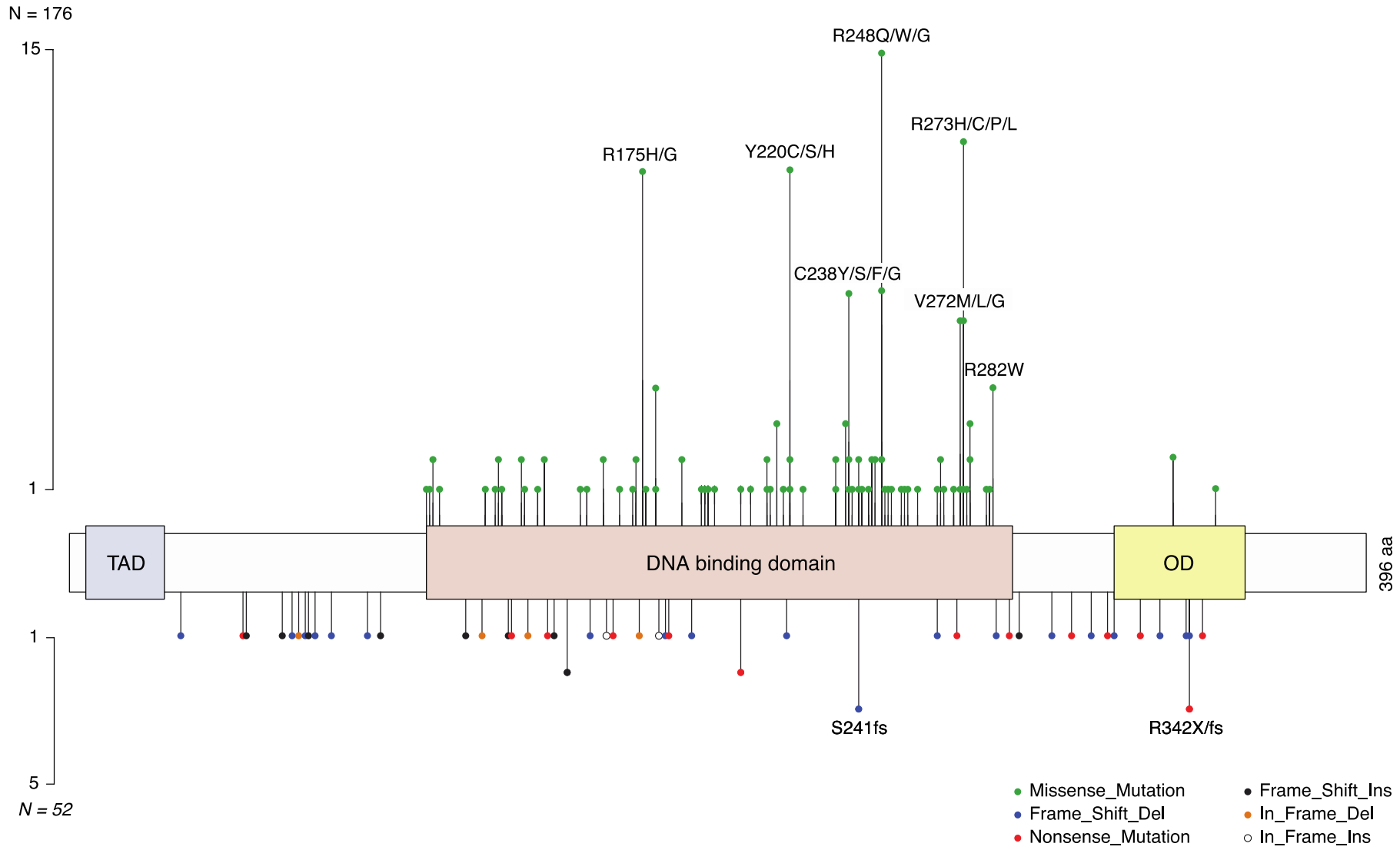


Figure S4.
Variant allele frequency of *TP53* mutations (A) and *TP53* variant allele frequency in relation to chromsome 17 aberrations and complex karyotype (B). *TP53* mutant AML/MDS-EB patients without an abnormality involving chromosome 17 (norm17) or complex karyotype (blue); *TP53* mutant AML/MDS-EB patients with norm17 and complex karyotype (orange) and *TP53* mutant AML/MDS-EB patients with an abnormality involving chromosome 17 (abn17) and complex karyotype (gray).

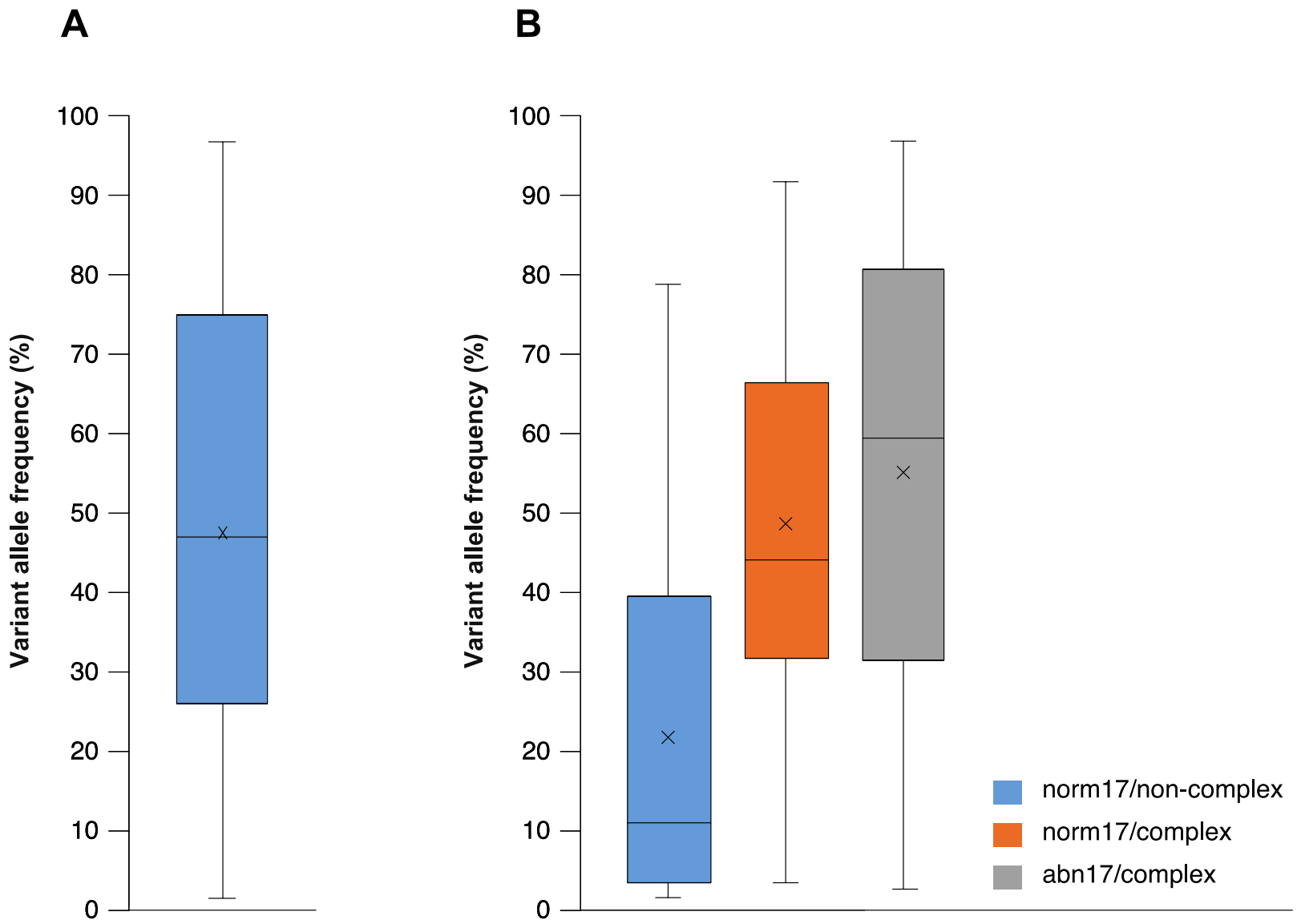


Figure S5.
Overall survival of *TP53* mutant allelic status (mono-allelic versus bi-allelic) in MDS-EB patients (n=44).

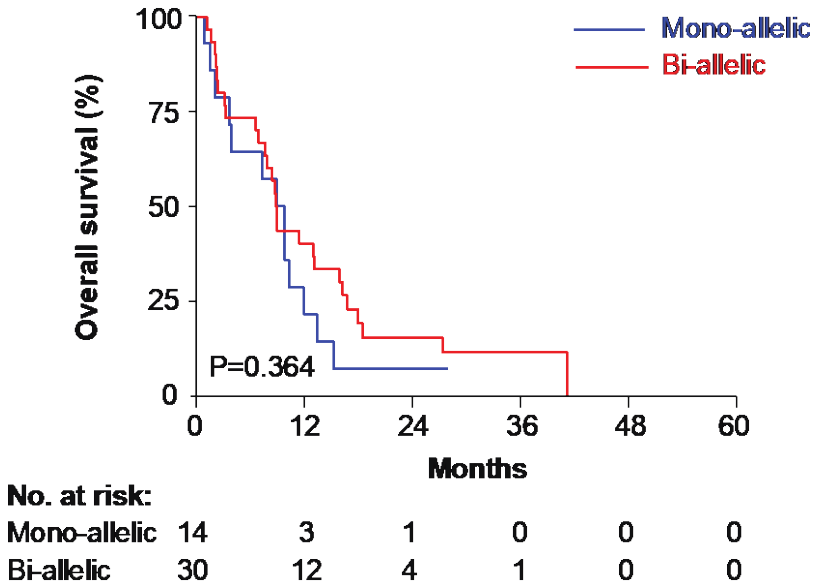
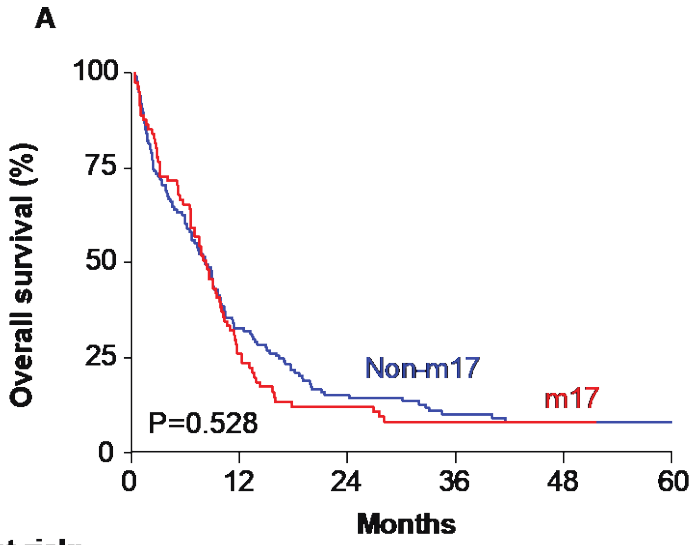
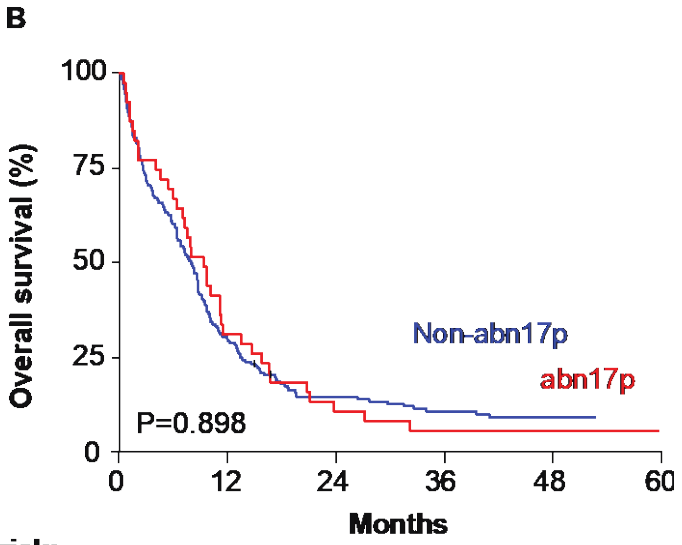


Figure S6.
Overall survival of mutant *TP53* AML/MDS-EB by chromosome 17p aberrations. Monosomy 17 (A) and abnormality chromosome 17p (B).



No. at risk:						
Non-m17	139	45	19	11	6	3
m17	81	20	9	4	3	2



No. at risk:						
Non-abn17p	181	53	24	14	8	5
abn17p	39	12	4	1	1	0

Figure S7.
Overall survival of mutant *TP53* AML/MDS-EB by individual concurrent mutations.

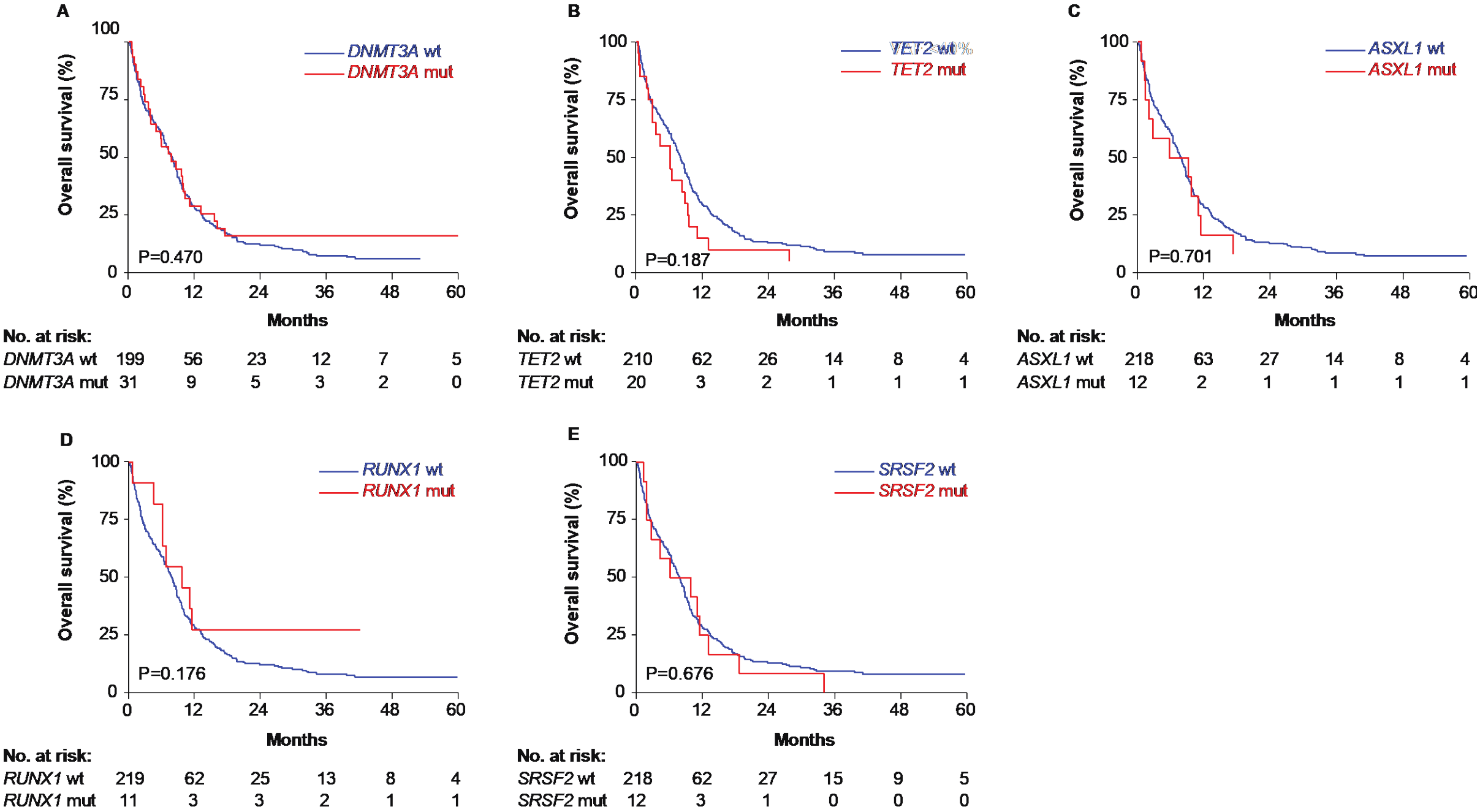
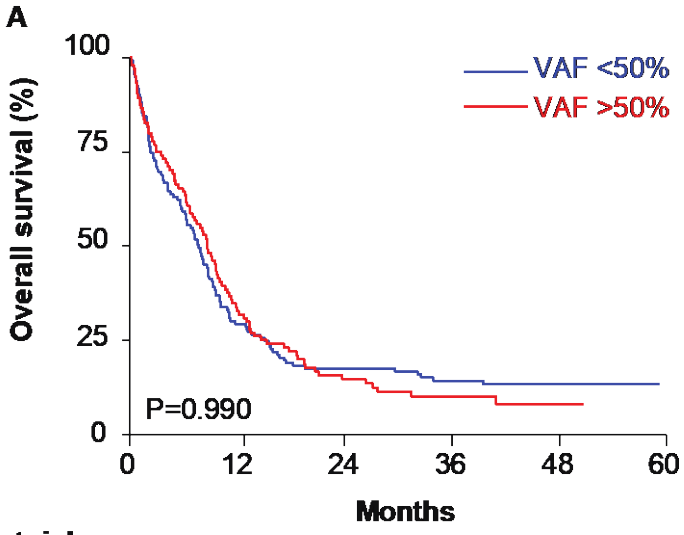
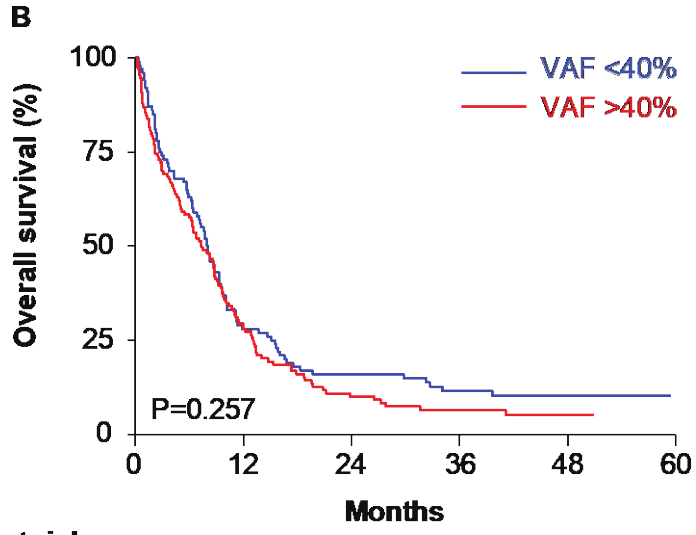


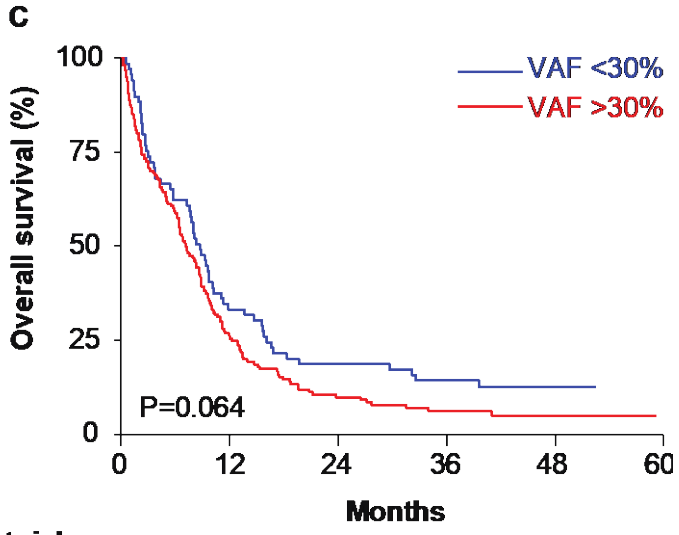
Figure S8.
Overall survival by mutant *TP53* variant allele frequency cut off.



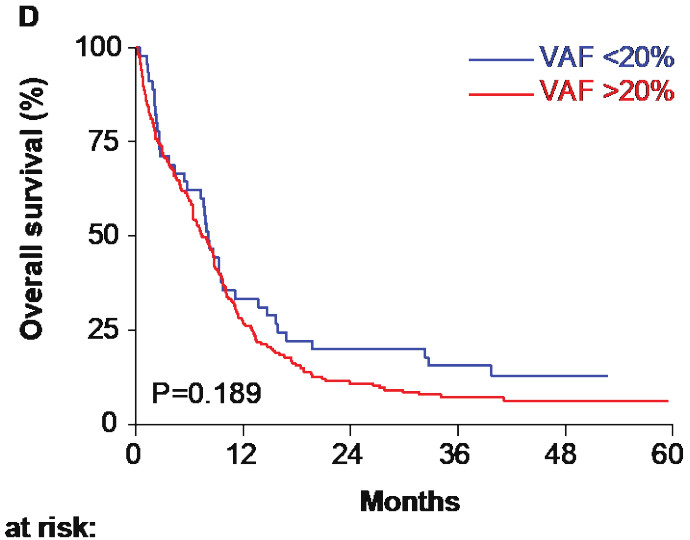
No. at risk:						
VAF <50%	130	34	18	11	7	4
VAF >50%	100	31	10	4	2	1



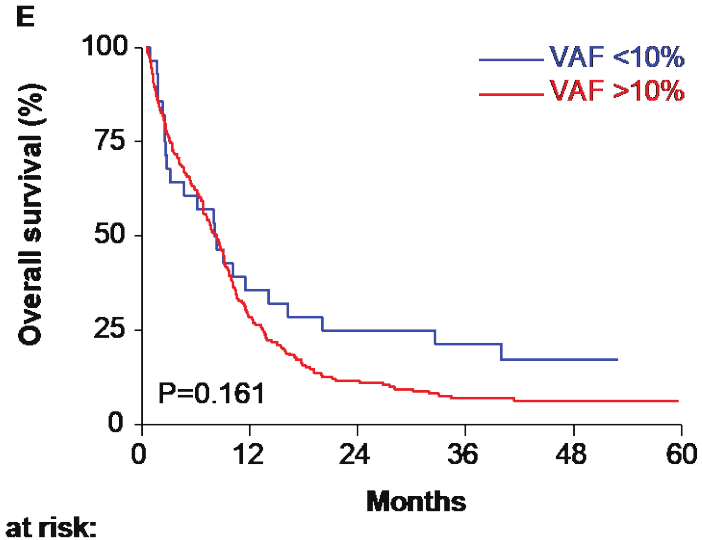
No. at risk:						
VAF <40%	100	28	16	9	6	4
VAF >40%	130	37	12	6	3	1



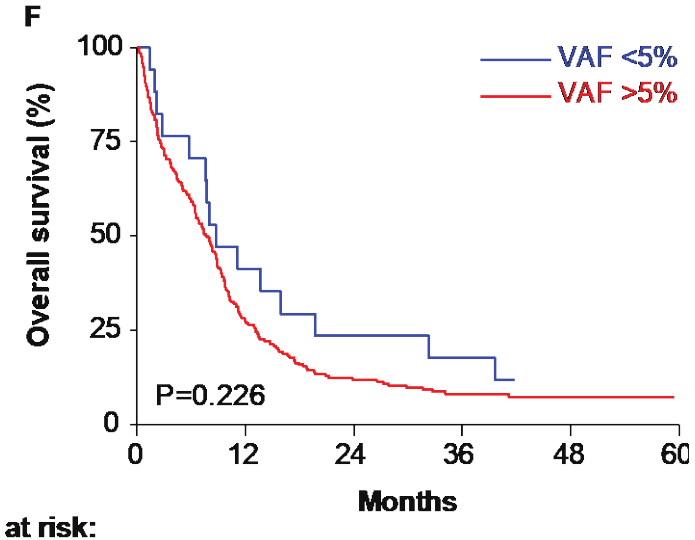
No. at risk:						
VAF <30%	69	23	13	8	5	4
VAF >30%	161	42	15	7	4	1



No. at risk:						
VAF <20%	45	15	9	6	4	3
VAF >20%	185	50	19	9	5	2



No. at risk:						
VAF <10%	28	10	7	5	3	2
VAF >10%	202	55	21	10	6	3



No. at risk:						
VAF <5%	17	7	4	3	1	1
VAF >5%	213	58	24	12	8	4