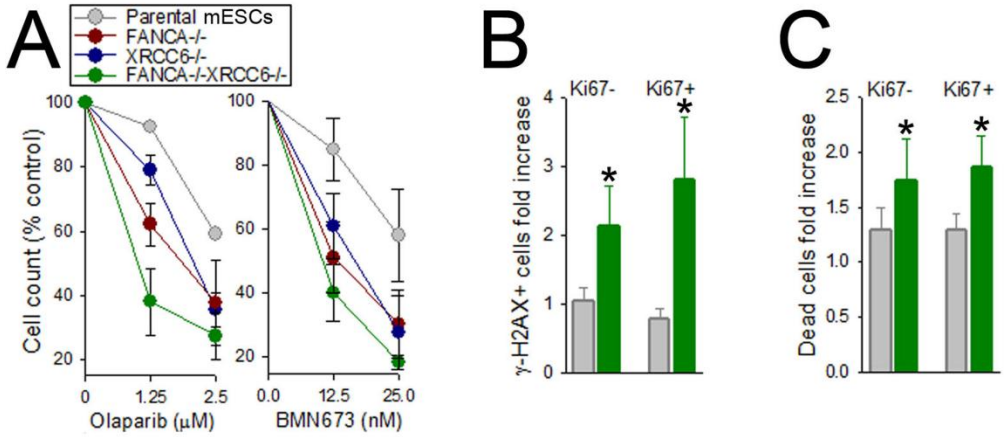
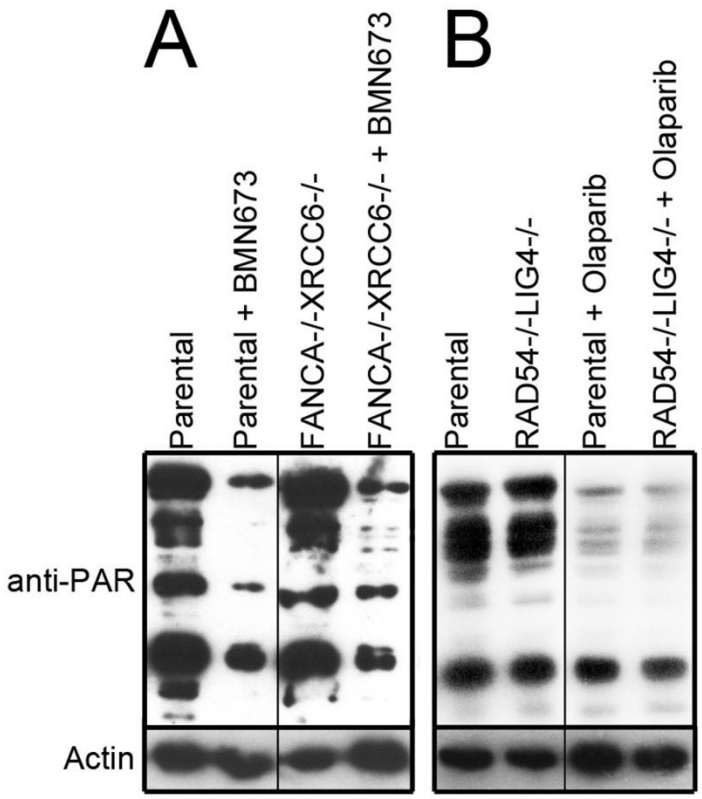
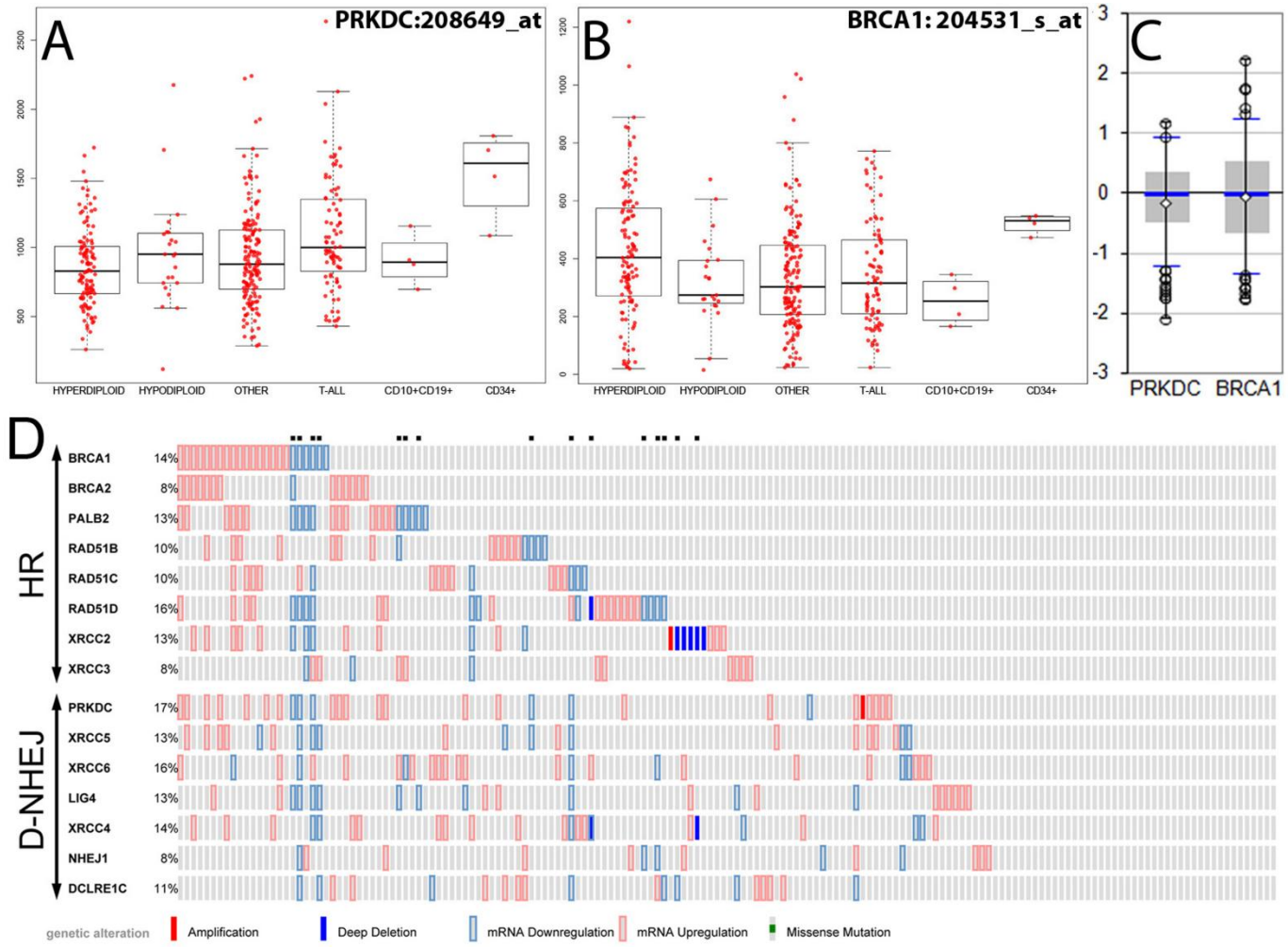


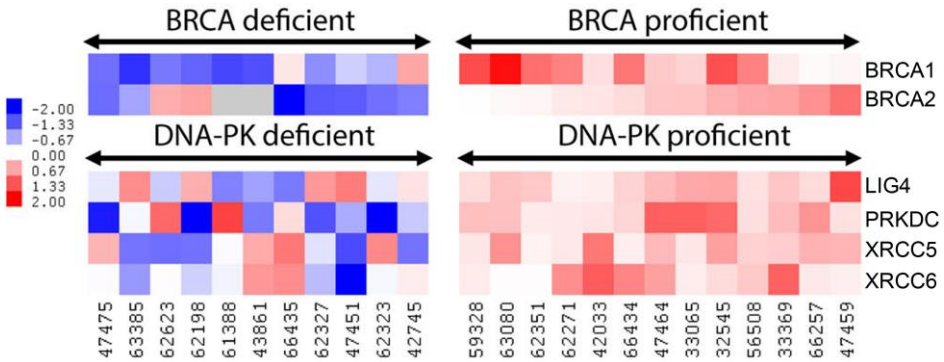
Supplementary Fig. 1. PARP1i elevated DSBs and triggered synthetic lethality in *FANCA-/-* and/or *XRCC6-/-* proliferating cells and in *XRCC6-/-* quiescent cells. (**A**) Sensitivity of the indicated cells to olaparib and BMN673. (**B, C**) Parental (grey bars) and *FANCA-/- XRCC6-/-* (green bars) mESCs treated with 12.5 nM BMN673 for 72 hours; (**B**) γ-H2AX –positive Ki67$^-$ and Ki67$^+$ cells; (**C**) Dead Ki67$^-$ and Ki67$^+$ cells. Results represent mean ± SD from 3 experiments; *p<0.05 in comparison to parental counterparts using Student t test.



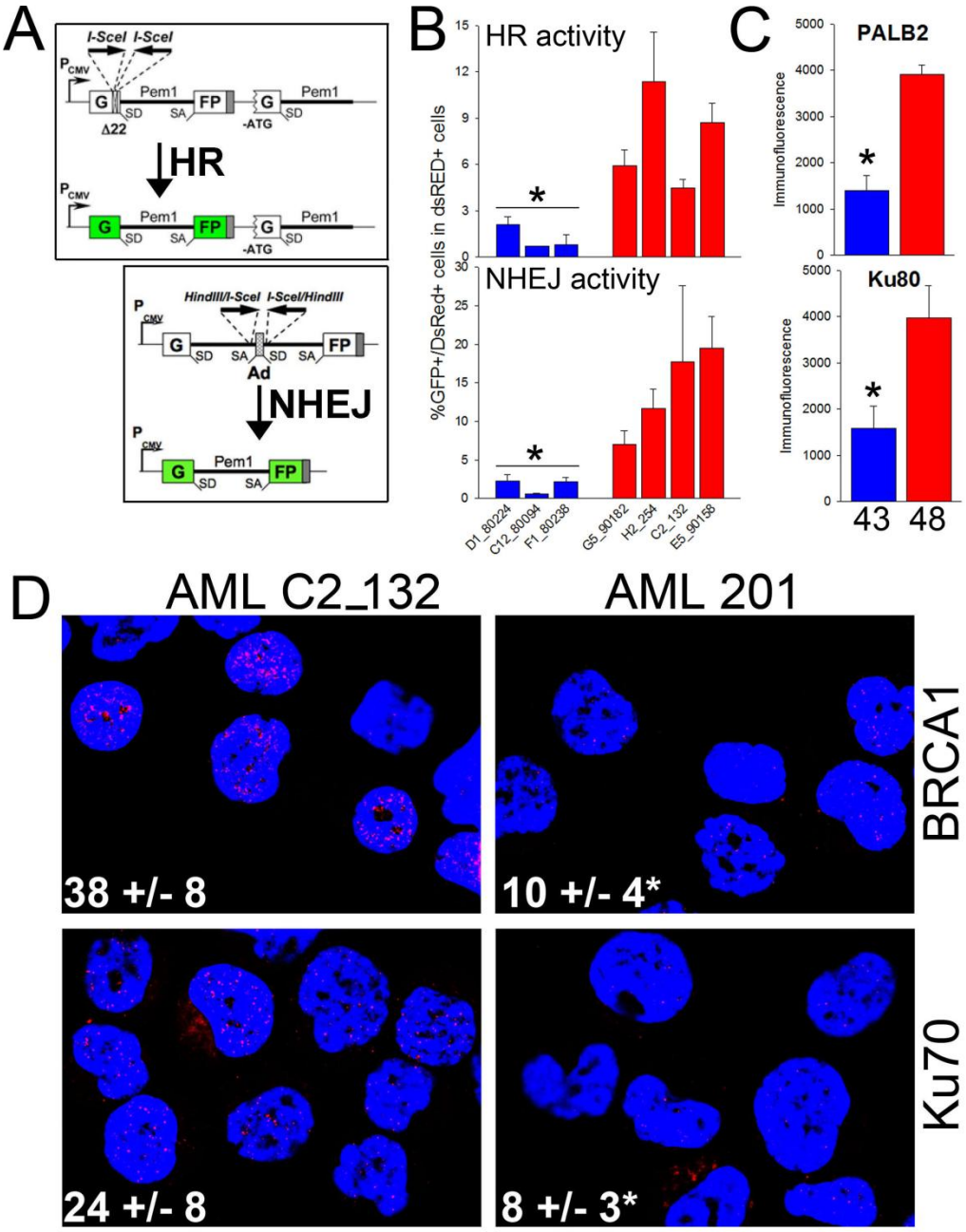
Supplementary Fig. 2. PARP1i downregulated PARylated proteins. (**A**) Parental and *FANCA-/-XRCC6-/-* mESCs, and (**B**) parental and *RAD54-/-LIG4-/-* Nalm-6 cells were treated or not with 0.3 μM olaparib or 3 nM BMN673 for 24 hours. Total cell lysates were analyzed by Western blot for PARylated proteins; actin served as loading control.



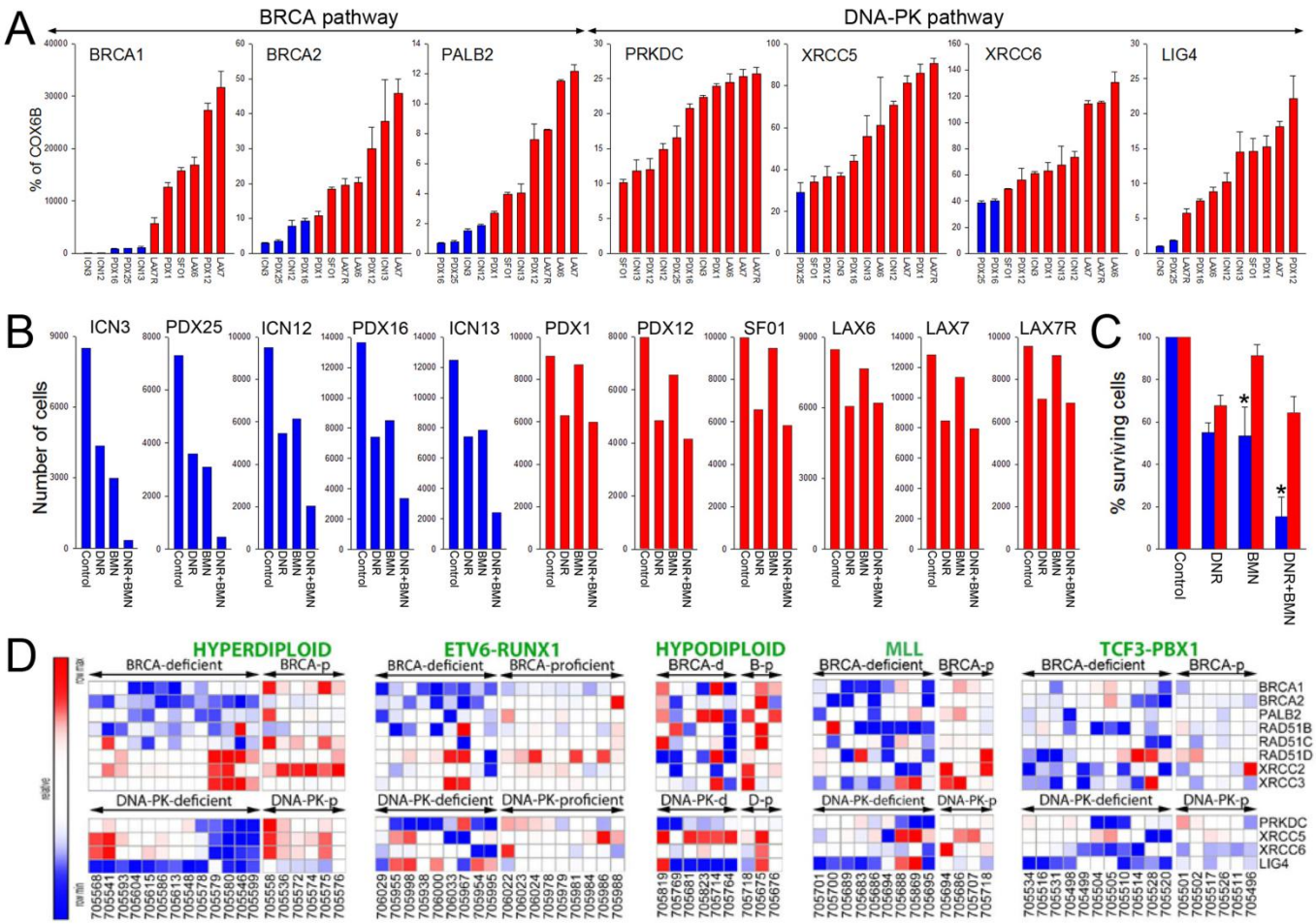
Supplementary Fig. 3. Wide-range expression levels of PRKDC and BRCA1 in ALLs and AMLs generate BRCA/DNA-PK deficient individual leukemias. Microarray analysis of the expression of PRKDC (**A**) and BRCA1 (**B**) in various types of B-ALL (from left to right): hyperdiploid, hypodiploid, other, in T-ALL, and in normal $CD10^{+}CD19^{+}$ cells and $CD34^{+}$ cells [Pediatric Cancer Genome Project (St. Jude Children's Research Hospital and Washington University; http://explore.pediatriccancergenomeproject.org]. Each red dot represents expression level of PRKDC and BRCA1 from individual patient. (**C**) Microarray analysis of PRKDC and BRCA1 expression in AMLs characterized by Figueroa and colleagues (*1*). (**D**) Analysis of TCGA database revealed individual AMLs displaying biomarkers of BRCA/DNA-PK deficiency. The indicated genes encoding proteins in HR and D-NHEJ pathways were included in to the analysis. Samples displaying putative BRCA/DNA-PK deficiency (deep deletion and Z-score threshold ≤-1.5) are marked by dots.



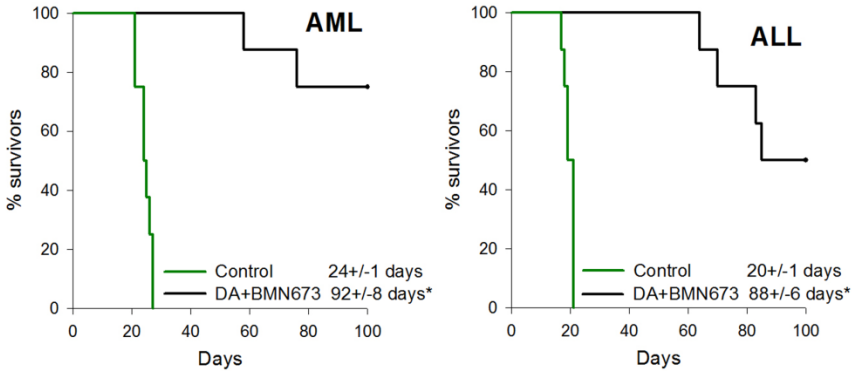
Supplementary Fig. 4. Microarray expression pattern analysis of the gens of BRCA and DNA-PK pathways in AML patients predicting BRCA/DNA-PK deficiency/proficiency. We identified 13 BRCA/DNA-PK deficient patients in microarrays from 436 AML patients (NCBI accession # GSE16432) using limited number BRCA and DNA-PK pathway genes with available data [clinical trials AMLSG HD98A and AMLSG HD98B (*2*)].



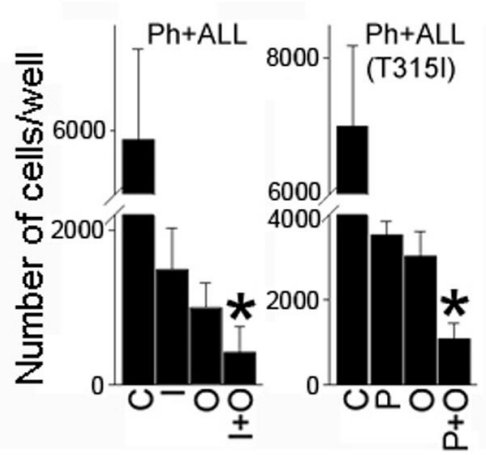
Supplementary Fig. 5. Functional assays of HR and NHEJ in BRCA/DNA-PK deficient and proficient cells. (**A**) HR and NHEJ reporter cassettes and repair products. (**B**) HR and NHEJ activities in BRCA/DNA-PK deficient (blue) and proficient (red) primary AML samples were detected as describe before with modifications (3-5). Two- to five million AML cells were nucleofected with 5 µg of I-SceI – linearized HR or NHEJ reporter plasmid and 2.5 µg of pDsRed plasmid using Nucleofector (Lonza; program U-008, Human CD34 Cell Nucleofector® Kit). HR or NHEJ event restores functional GFP expression. After 72 hours the percentage of GFP+/DsRed+ cells in DsRed+ cells was analyzed by flow cytometry to assess HR and NHEJ activity. Results represent mean ± SD from triplicates/sample; *p<0.001 in comparison to all BRCA/DNA-PK-positive samples. (**C**) Flow cytometry analysis of DSB repair proteins in individual ALLs (#43 and #48) predicted to be BRCA/DNA-PK deficient (blue) or proficient (red). Results represent mean ± SD from 3 measurements/sample. (**D**) Representative nuclear staining (DAPI) for BRCA1 and Ku70 foci in BRCA/DNA-PK deficient AML 201 and BRCA/DNA-PK proficient AML C2-132 cell samples. Foci were detected as described before (3, 6). Results represent mean ± SD number of foci/nucleus in 10 cells displaying ≥5 foci/cell per experimental group. *p≤0.001 using Student t test.



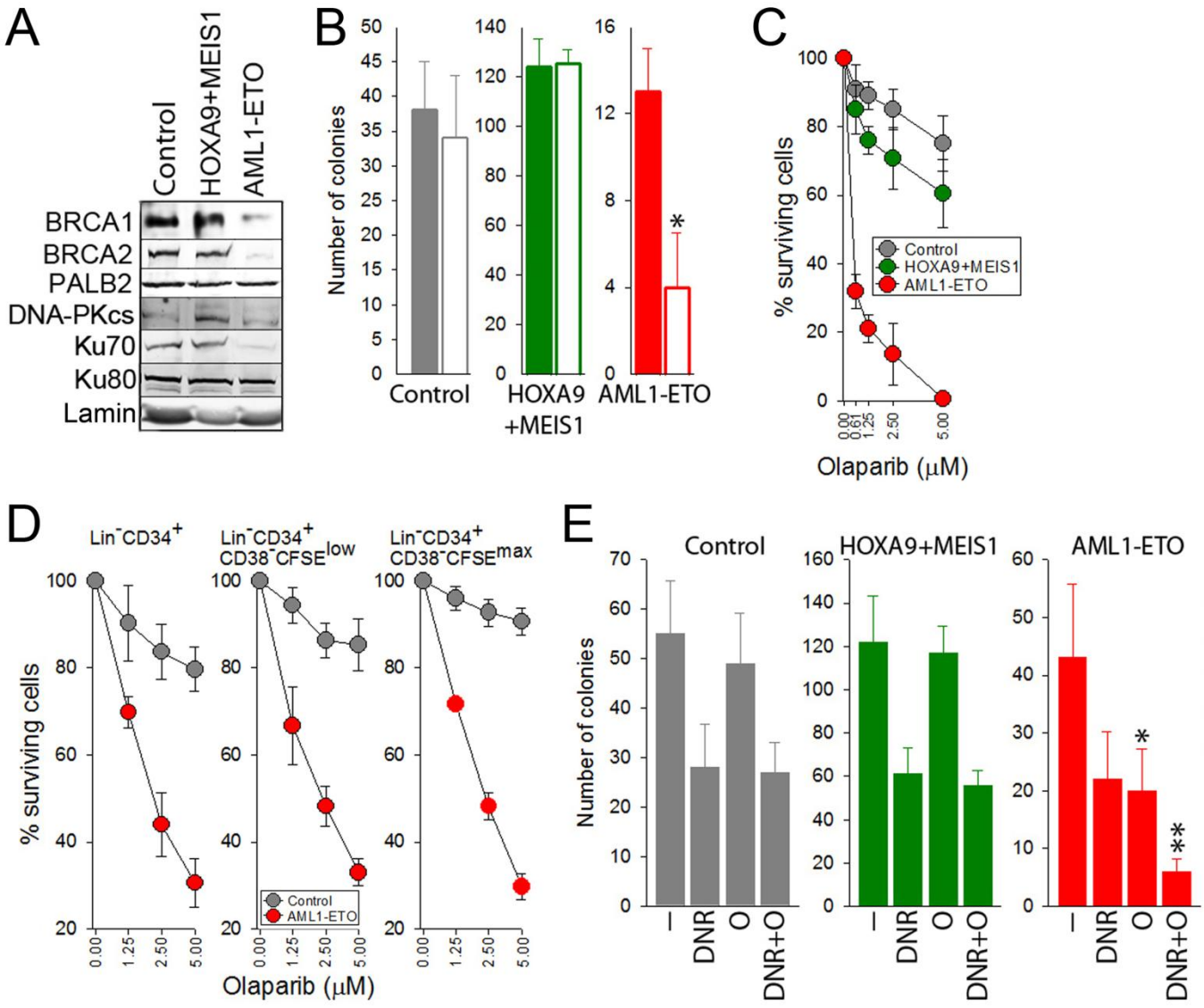
Supplementary Fig. 6. RT-qPCR and mRNA microarray expression pattern analyses of the genes of BRCA and DNA-PK pathways in Philadelphia chromosome-negative B-ALLs from individual patients predict BRCA/DNA-PK deficiency/proficiency. (**A**) Primary cells from 11 individual B-ALL samples were tested simultaneously by RT-qPCR for expression levels of genes in BRCA and DNA-PK pathways, which were then normalized against a housekeeping gene such as COX6B. The most dramatic differences between individual samples were observed in expressions of BRCA1, PALB2 and BRCA2 from the BRCA pathway (546, 17, and 15 -fold difference between the highest and lowest levels, respectively), and also of LIG4 from the DNA-PK pathway (20-fold) whereas PRKDC, XRCC5 and XRCC6 demonstrated only 2-3 –fold difference. Samples, in which low expression of BRCA/DNA-PK genes was associated with BMN673 sensitivity or resistance (see panel **B**), are marked in blue and red, respectively. (**B**) Sensitivity to 1 μg/ml DNR, 25 nM BMN673, or DNR + BMN673 of the primary ALL xenograft cells from BRCA and/or DNA-PK deficient (blue bars) and BRCA and/or DNA-PK proficient (red bars) samples. (**C**) Cumulative percentages of cells from samples examined in panel **B**; $p<0.001$ in comparison to corresponding BRCA/DNA-PK proficient samples using Student t test. (**D**) Microarray analyses of gene expression patterns in 308 Philadelphia chromosome-negative B-ALL samples [NCBI accession # GSE33315 (7)] predicted BRCA and DNA-PK deficient phenotype in 13 of 116 hyperdiploid, 9 of 99 ETV6-RUNX1, 6 of 23 hypodiploid, 9 of 30 MLL-rearranged, and 11 of 40 TCF3-PBX1 patient samples. B-p and BRCA-p = BRCA proficient; D-p and DNA-PK-p = DNA-PK proficient.



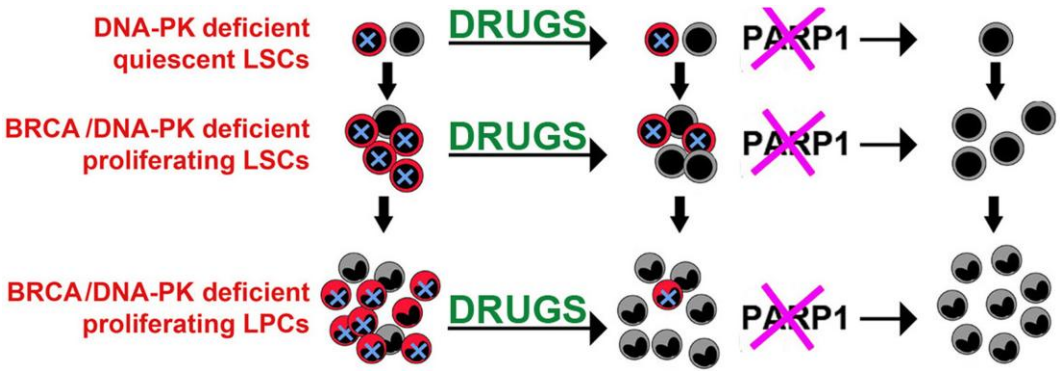
Supplementary Fig. 7. DA+BMN673 eliminated LSCs from BRCA/DNA-PK deficient AML and ALL PLXs in vivo. Sub-lethally irradiated NSG mice were injected intravenously with 2 x 10^6 bone marrow cells harvested from NSG mice carrying BRCA/DNA-PK deficient AML and ALL PLXs, which were untreated or treated with DA+BMN673 (see Figure 7). Survival curves and MST ± SE (8 mice/group); *$p<0.001$ using Kaplan-Meier LogRank test.



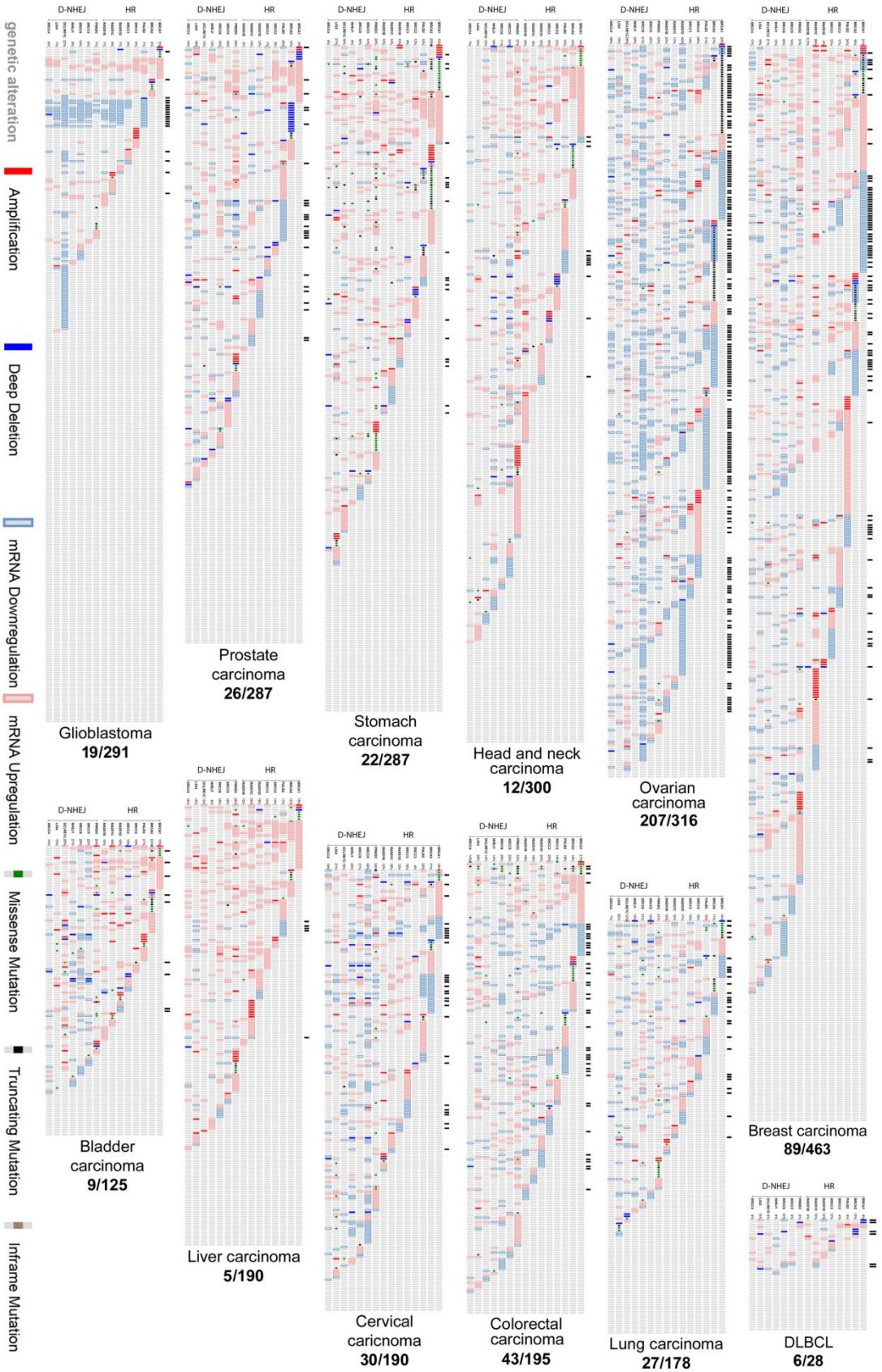
Supplementary Fig. 8. PARP1i olaparib combined with TKI exerted synergistic anti-Ph+ALL effect. Leukemia cells from patients with not mutated BCR-ABL1 kinase [Ph+ALL; n=3] and these from patients at relapse with T315I mutation [Ph+ALL(T315I); n=3] were untreated (C) or treated with 1μM imatinib (IM), 12.5nM ponatinib (P), 2.5 μM olaparib (O) and a combination of IM + O or P + O. Living cells were counter after 5 days using Trypan blue; *$p<0.02$ in comparison to cells treated with individual drugs using two-way ANOVA.



Supplementary Fig. 9. Leukemia expressing AML1-ETO display BRCA/DNA-PK deficiency and are sensitive to PARP1i-mediated dual cellular synthetic lethality. (**A-C**) Non-transformed GFP+ mBMCs (Control) and those expressing AML1-ETO, and HOXA9 + MEIS1 were examined. (**A**) Western analysis; (**B**) Colonies from *Parp1*+/+ (solid bars) and *Parp1*-/- (transparent bars) mBMCs (triplicate experiment); *p=0.03 using Student t test. (**C**) Cells surviving olaparib treatment (triplicate experiment). (**D**) Lin^-CD34^+ LPCs/HPCs, $Lin^-CD34^+CD38^-CFSE^{max}$ quiescent and $Lin^-CD34^+CD38^-CFSE^{low}$ proliferating AML LSCs expressing AML1-ETO (n=3) and HSCs from healthy donors (Control, n=3) surviving olaparib treatment. (**E**) Colonies from GFP+ mBMCs (Control) and these expressing HOXA9 + MEIS1 or AML1-ETO, untreated (-) and treated with 1μg/ml DNR, 0.6125μM olaparib (O), or DNR+O (triplicate experiment); *p<0.02 in comparison to control using Student t test; **p≤0.006 in comparison to DNR and O using Student t test adjusted to multiple comparisons.



Supplementary Fig. 10. Proposed model of dual cellular synthetic lethality triggered by PARP1i. Leukemia cells (red), in contrast to normal counterparts (grey), accumulate numerous spontaneous and drug-induced DSBs (light blue cross). Currently approved drugs eliminate a bulk of proliferating LSCs/LPCs, but cannot eradicate drug-refractory quiescent LSCs and drug-resistant LSCs/LPCs. These cells could be eradicated by PARP1i causing dual cellular synthetic lethality in DNA-PK –deficient quiescent LSCs and BRCA/DNA-PK –deficient proliferating LSCs/LPCs, whereas normal cells will be spared.



Supplementary Fig. 11. BRCA/DNA-PK deficiency detected in various tumor types from individual patients in TCGA database. Numbers of BRCA/DNA-PK deficient samples/total numbers of samples are shown. Samples displaying biomarkers of BRCA/DNA-PK deficiency are marked by dots.

Supplementary Table 1. Clinical annotation for CML cases.

Diagnosis	ID	Molecular lesion	Karyotype
CML-CP	142	*BCR-ABL1*	46,XY,t(9;22)(q34;q11)
CML-CP	143	*BCR-ABL1*	46,XY,t(9,22)(q34;q11)
CML-CP	145	*BCR-ABL1*	46,XY,t(9;22)(q34;q11)
CML-CP	146	*BCR-ABL1*	46,XY,t(9;22)(q34;q11)
CML-CP	155	*BCR-ABL1*	Not available
CML-CP	158	*BCR-ABL1*	Not available
CML-CP	170	*BCR-ABL1*	46,XX,t(9;22)(q34;q11)
CML-CP	171	*BCR-ABL1*	46,XY,t(9;22)(q34;q11)
CML-CP	178	*BCR-ABL1*	Not available
CML-CP	179	*BCR-ABL1*	Not available
CML-AP	51	*BCR-ABL1*	Not available
CML-AP	52	*BCR-ABL1*	Not available
CML-AP	60	*BCR-ABL1*	Not available

Supplementary Table 2. Clinical annotation for AML cases.

Diagnosis	ID	Molecular lesion	Karyotype	Flow cytometry
AML	201	*Not available*	Del12(q13-q15)	CD13, CD33, CD34, CD117
AML	687	*AML1-ETO*	46,XY,t(8;21)(q22;q22)	CD10-, CD13 dim +, CD14-, CD15 large subset +, CD19 dim +, CD20-, CD33+, CD34+, CD64-, CD79 dim +, HLA-DR+, TdT dim +
AML	1587	*AML1-ETO*	46,XY,t(8;21)(q22;q22),del(13)(q12q22)	CD10-, CD13+, CD14-, CD15 var +, CD19 dim +, CD20-, CD33+, CD34 dim +, CD64 dim +, CD79-, HLA-DR+, TdT-
AML	2281	*AML1-ETO*	6,XX,t(8;21)(q22;q22),del(9)(q22)	CD10 variable +, CD13+, CD15-, CD19+, CD20 small +/-, CD33 dim variable +, CD34+, CD79+, HLA-DR+, TdT subset +
AML	132	*FLT3(wt), NPM(wt)*	Not available	Not available
AML	254	*FLT3(wt), NPM(wt)*	Not available	Not available
AML	8127	*Not available*	Not available	Not available
AML	9307	*FLT3(TKD), NPM(na)*	Not available	Not available
AML	80088	*FLT3(wt), NPM(mut)*	Not available	Not available
AML	80094	*NPM(wt), FLT3(wt)*	Not available	Not available
AML	80224	*FLT3(wt), NPM(mut)*	Not available	Not available
AML	80238	*FLT3(wt), NPM(mut)*	Not available	Not available
AML	90158	*Not available*	Not available	Not available
AML	90182	*FLT3(wt), NPM(wt)*	Not available	Not available
AML	100112	*FLT3(wt), NPM(wt)*	Not available	Not available
AML	747899	*Not available*	Not available	Not available

ID# 201 = PLX

Supplementary Table 3. Clinical annotation for ALL cases (PLXs).

Diagnosis	ID	Molecular lesion	Karyotype	Flow cytometry
B-ALL	ICN3	*MLL-AF4*	Not available	Not available
B-ALL	ICN12	*E2A-PBX1*	t(1;19)(q23;p13)	Not available
B-ALL	ICN13	*MLL-AF4*	t(4;11)(q21;q23)	Not available
B-ALL	LAX6	*IGH-TCRB*	t(7;14)(q34;q32)	Not available
B-ALL	LAX7	*Not available*	Not available	Not available
B-ALL	LAX7R	*Not available*	Not available	Not available
B-ALL	SFO1	*Not available*	Not available	Not available
B-ALL	PDX1	*Not available*	Not available	Not available
B-ALL	PDX12	*Not available*	Not available	Not available
B-ALL	PDX16	*MLL-AF4*	46,XY,t(4;11)(q21;q23)[1]/46,sl,t(9;14)(p?13;q?11.2)[12]/46,sdl1,+X,-der(14)t(9;14)[3]/47, sdl1,+X,add(9)(p2?2)[6]/46,XY[2]	Not available
B-ALL	43	*Not available*	11q23	Not available
B-ALL	48	*Not available*	Not available	Not available
B-ALL	LAX9	*BCR-ABL1*	t(9;22)(q34;q11)	Not available
B-ALL	PDX2	*BCR-ABL1*	Not available	CD10, CD19, CD22, CD34, cytoplasmic CD79a,dim CD123, HLA-DR, and TdT+
B-ALL	TXL3	*BCR-ABL1*	t(9;22)(q34;q11)	Not available
B-ALL	BLQ1	*BCR-ABL1 (T315I)*	der(9), der(22)	Not available
B-ALL	BLQ5	*BCR-ABL1 (T315I)*	der(9), der(22)	Not available
B-ALL	LAX2	*BCR-ABL1 (T315I)*	t(9;22)(q34;q11)	Not available

Supplementary Table 4. Clinical annotation for t-MDS/AML cases.

Primary diagnosis	ID	Karyotype at t-MDS	Morphology at t-MDS
HD	L5021	del(5), del(18), +(2), +(3)	AML w/ multilineage dysplasia
Breast carcinoma	L5034	del5q, +8, +11	AML
Bladder carcinoma	L5030	normal	AML 40-50% blasts, hypercellular marrow (90%)
MM	L5047	monsomy 2, 11q13	MDS, hypercellular (40%), dysplastic megakaryocytes
NHL	L5043	del7q	AML 24% blasts, hypocellular marrow (20%)
NHL	L5035	del(5q),t(1;2), t(12;15), -2, -3, -21	MDS, RAEB2, 16.5% blasts
Ovarian carcinoma	L5168	del18q	MDS, hypercellular (59%), trilineage dyspoisis excess blasts (16%)
Cervical and rectal carcinoma, NHL	L5251	monosomy 5, monosomy 7	AML, hypercellular (50%), 27% blasts
NHL	L5075	normal	MDS, RAEB2, 15% blasts
NHL	L5164	inversion 1 , +Y	MDS
Breast carcinoma	L5033	t(11;17)(q23;25), trisomy 6,8,12,18,19,20	AML, 84% blasts
Breast carcinoma	L5250	monosomy 7, del(5q)	MDS, normocellular, low-grade
NHL	L5166	del(20q)	normal, thrombocytopenia, anemia
HD/HIV	L5258	del7q	MDS, normocellular, low-grade
Cervical, rectal carcinoma	L5232	del7q, monosomy 12	MDS, 6% blasts
NHL	L5069	monosomy 7	normal, anemia, thrombocytopenia
CLL	L5261	monosomy 5, del7q, del4p, t(12;14)	MDS, <5% blasts, erythroid hyperplasia, dysmegakaryopoiesis
NHL	L5217	del5q, del7q, del20q	MDS, normocellular
NHL	L5071	monsomy 7, t(2;19), t(3;9), +20p	MDS, mild dyserythropoiesis
Prostate, Kidney carcinoma	L5064	del3, -5, del6, del 16, del 17, +22,	MDS, multilineage dysplasia, <5% blasts
APL	L5144	del 7q	MDS, low-grade
AML	L5222	t(2;3)(p23;q29)	MDS, myeloid left shift
ALL	L5080	t(7;20), del 20q	MDS, hypercellular (>95%), 3-5% blasts

HD = Hodgkin disease, MM = multiple myeloma, NHL = non-Hodgkin lymphoma, CLL = chronic lymphocytic leukemia, APL = acute promyelocytic leukemia, AML = acute myeloid leukemia, ALL = acute lymphoblastic leukemia

Supplementary Table 5. Primer sequences for RT-qPCR analysis of B-ALLs

Gene	**Sense primer (5'-3')**	**Antisense primer (5'-3')**
PALB2	GGGACTTACTTCTCGGTCAGTGTAC	CGACCATTTCACAAAAGACCAA
BRCA1	GGAGGTCAGGAGTTCGAAACC	ACCGGCTAATTTCTGTATTTTTAGTAGAG
BRCA2	ACCTGTTAGTCCCATTTGTACATTTG	CACAACTCCTTGGTGGCTGAA
RAD51	TTCAGGCCAGTGTGGTGTCTT	TGGGCTCAAGCGATCCA
PRKDC	CTGATGGACCAGGCAACAGA	TCCAGGGCTCCCATCCTT
XRCC5	TGTGTTGAGCAAGCAGTAGCATT	AGTCCGTCCTTACCCATGGTT
XRCC6	TGGCCTTGGATTTGATGGA	CAGTCTTTTATTCATTGCTTCAACCT
LIG4	ATGGCTTCTCTGATTGCTGATTT	CGGTGTGGCGTCGAAAC
COX6b	AACTACAAGACCGCCCCTTT	GCAGCCAGTTCAGATCTTCC

Supplementary Table 6. Primer sequences for RT-qPCR analysis of t-MDS/AML.

Hs00984230_m1	**B2M**
Hs01556193_m1	**BRCA1**
Hs00609073_m1	**BRCA2**
Hs02758991_g1	**GAPDH**
Hs01866071_u1	**LIG4**
Hs00227120_m1	**NHEJ1**
Hs00226617_m1	**PALB2**
Hs04195439_s1	**PRKDC**
Hs00427442_m1	**RAD51C**
Hs00979545_g1	**RAD51D**
Hs03044154_m1	**XRCC2**
Hs00193725_m1	**XRCC3**
Hs00897854_m1	**XRCC5**
Hs01922655_g1	**XRCC6**

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