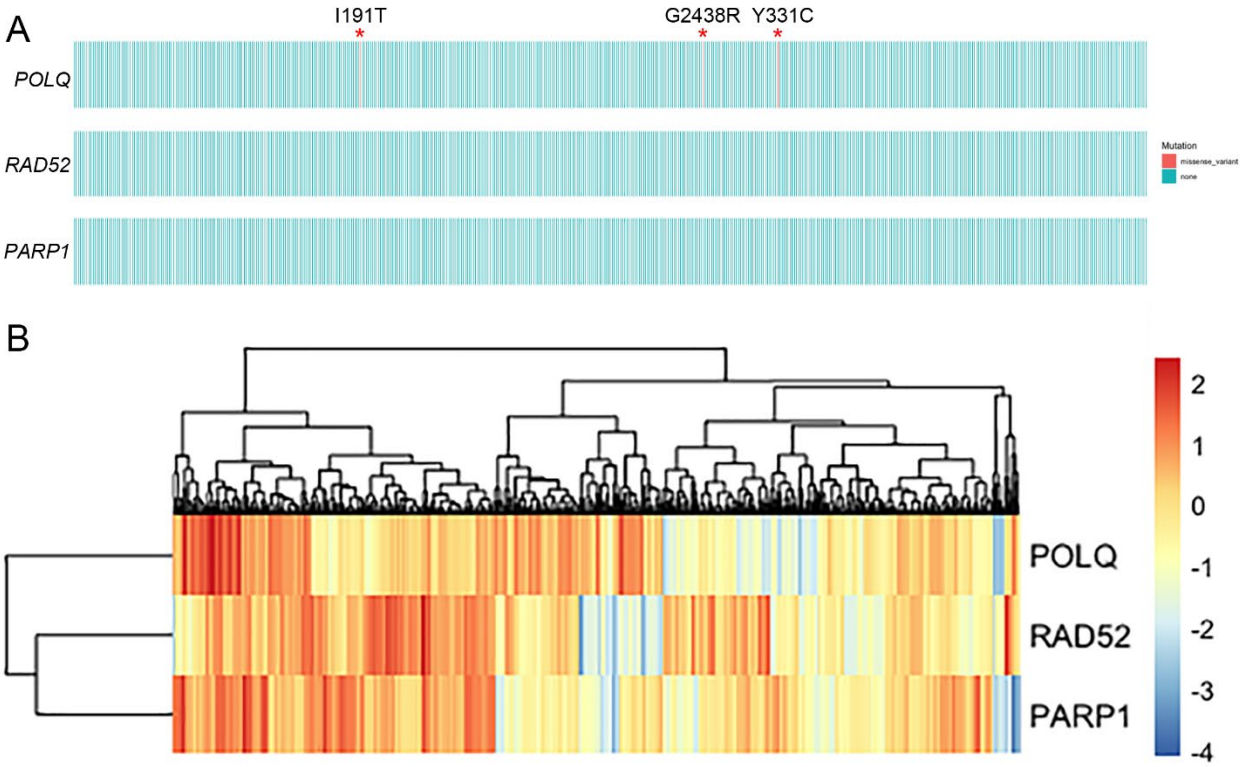
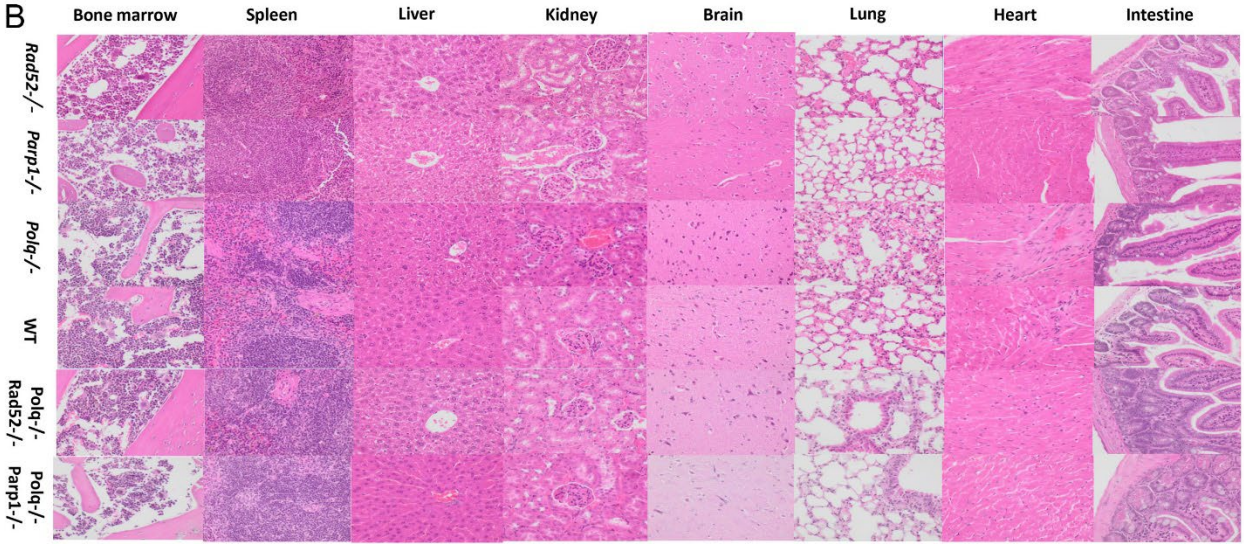


Supplementary Figure 1. Genetic aberrations and mRNA expression variabilities of *POLQ*, *PARP1* and *RAD52* do not coexist in 519 AML samples from the BEAT-AML cohort (https://www.vizome.org/aml/). (**A**) Matrix showing mutations in *POLQ*, *RAD52*, and *PARP1* genes. (**B**) Genewise-scaled variance stabilizing transformation (VST) normalized gene expression of *POLQ*, *RAD52*, and *PARP1*.

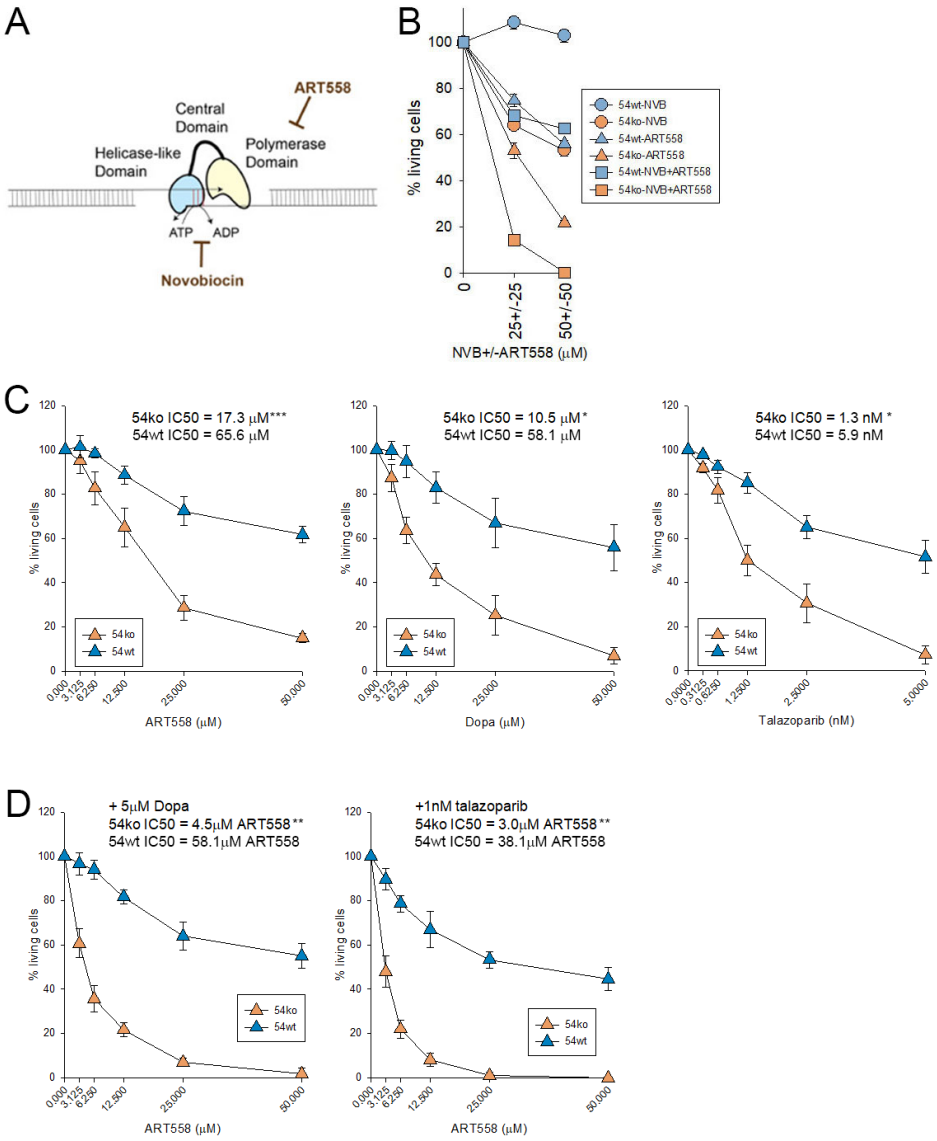
A

Tissue	Parameter	Unit	+/+ (n=6)		Polq-/- (n=6)		Parp1-/- (n=4)		Rad52-/- (n=3)		Polq-/-;Parp1-/- (n=4)		Polq-/-;Rad52-/- (n=4)	
			Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev
Peripheral blood	WBC	K/uL	11.773	3.256	13.433	4.403	14.747	3.279	13.047	2.885	14.140	7.691	10.895	3.647
	NE#	K/uL	3.400	2.007	3.378	1.040	3.173	1.141	2.753	0.560	3.713	3.679	3.903	2.474
	LY#	K/uL	7.513	1.211	9.268	3.024	9.693	1.259	8.727	1.867	9.355	3.298	5.963	1.727
	MO#	K/uL	0.570	0.170	0.598	0.318	0.847	0.263	0.690	0.176	0.605	0.308	0.490	0.194
	EO#	K/uL	0.253	0.153	0.145	0.110	0.833	0.498	0.680	0.208	0.363	0.448	0.385	0.348
	BA#	K/uL	0.085	0.053	0.043	0.027	0.363	0.185	0.197	0.116	0.105	0.157	0.155	0.162
	RBC	M/uL	10.253	0.478	9.723	0.433	8.947	0.690	9.547	0.241	9.553	0.440	10.153	0.683
	HB	g/dL	12.900	0.231	12.450	0.812	13.433	0.153	12.633	0.833	12.625	1.162	13.975	2.087
	HCT	%	45.917	2.250	46.950	3.256	47.533	2.065	49.033	5.258	47.825	8.357	55.525	8.446
	MCV	fL	44.783	0.100	48.283	2.150	53.300	3.666	51.433	6.700	49.950	6.575	54.550	5.884
	MCH	Pg	12.600	0.557	12.817	0.588	15.100	1.389	13.267	1.234	13.225	0.750	13.725	1.239
	PLT	K/uL	701.667	54.580	896.333	142.813	645.333	111.159	690.667	234.180	785.500	75.677	752.750	136.133
	Gr1+	% PBL	10.620	4.236	11.972	4.006	7.225	2.138	12.620	5.054	13.000	5.650	7.825	0.895
	Mac1+	% PBL	26.600	15.387	21.550	5.995	15.925	12.170	21.660	4.133	28.033	5.416	26.375	8.548
	CD3+	% PBL	26.600	15.897	25.225	7.104	30.825	7.454	33.880	8.175	21.233	5.772	24.125	15.923
	B220+	% PBL	31.160	9.036	27.875	4.647	24.050	6.153	29.680	6.855	28.700	3.905	24.075	2.587
Bone marrow	Lin^-cKit^+	% of Lin^-	19.232	2.413	18.74833	4.117866	17.150	8.613	17.867	0.802	16.375	1.410	16.875	3.308
	Lin^-Sca1^+	% of Lin^-	17.065	1.443	14.36333	4.924882	16.225	9.896	14.433	6.512	16.725	6.278	18.725	8.732
	$Lin^-cKit^+Sca1^+$	% of Lin^-	4.200	1.378	2.906667	1.229661	5.400	5.095	3.467	1.250	3.825	1.382	5.200	3.342

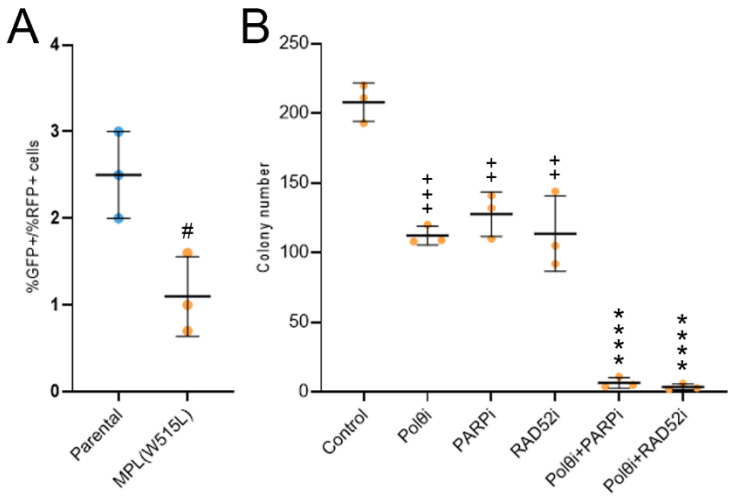
B



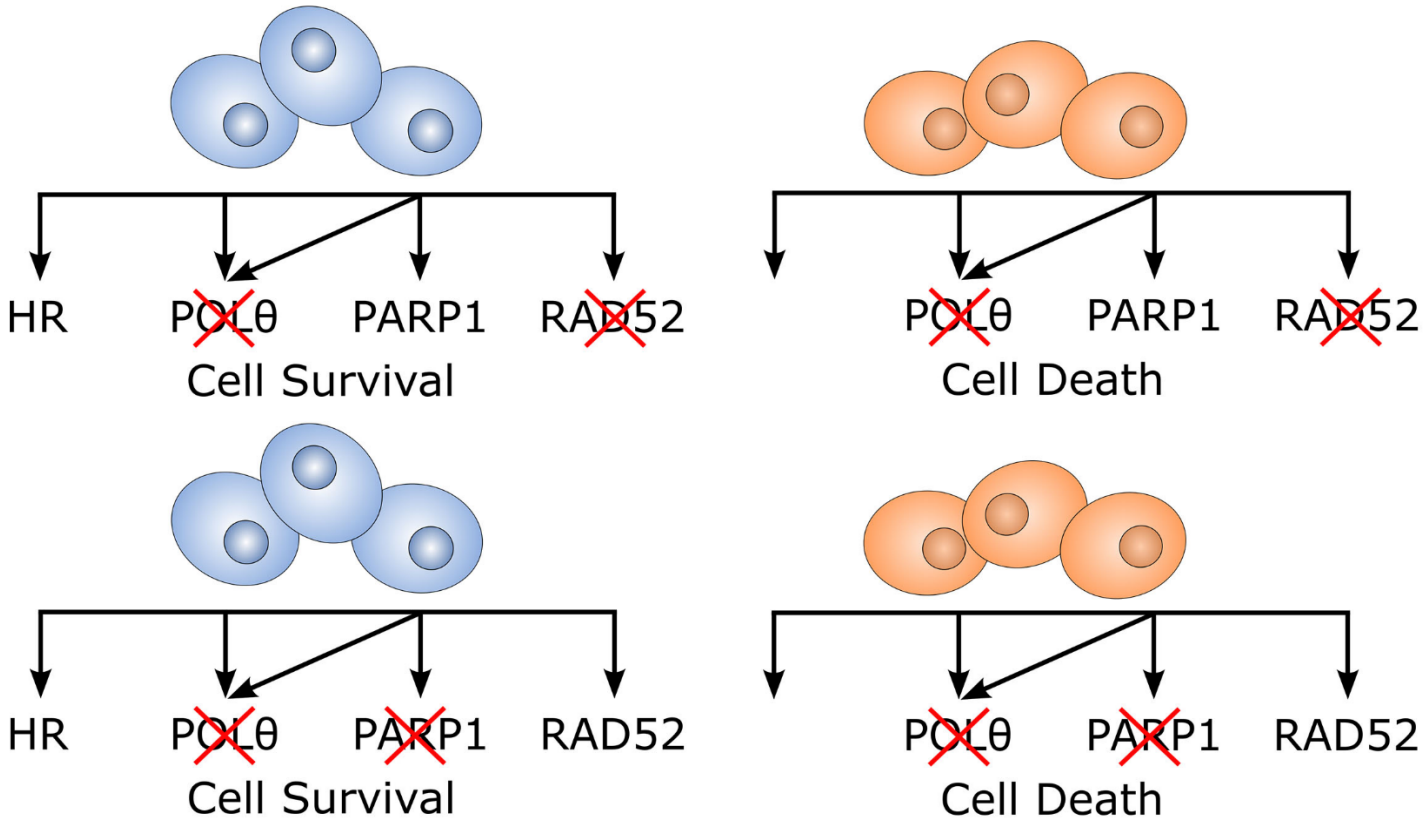
Supplementary Figure 2. Phenotype of *Polq-/-;Parp1-/-* and *Polq-/-;Rad52-/-* mice. (**A**) Peripheral blood and bone marrow parameters in 3-4 months old male and female mice (n = number of mice/genotype). (**B**) H&E-stained tissue sections (bone marrow, spleen, liver, kidney, brain, lung, heart, and bowel) with no specific pathologic changes (40x).



Supplementary Figure 3. Simultaneous inhibition of Polθ helicase and DNA polymerase activity exerted synergistic effect against *RAD54-/-* leukemia cells. (**A**) A scheme illustrating how novobiocin (NVB) and ART558 inhibit Polθ. (**B-D**) Nalm6 (54wt) and Nalm6-*RAD54-/-* (54ko) cells were treated for 72 hrs with the indicated concentrations of: (**B**) novobiocin (NVB) and ART558. (**C**) ART558, 6-hydroxy-DL-dopa (Dopa), talazoparib, and (**D**) ART558 + Dopa and ART558 + talazoparib. Results show mean % ± SD of living cells ± SD when compared to untreated cells (**B, C**) and cells treated with the indicated concentrations of Dopa or talazoparib (**D**). IC50 were calculated using Excel. *, **, *** in comparison to 54wt.



Supplementary Figure 4. Targeting Polθ + PARP and Polθ + RAD52 induced dual synthetic lethality against HR -deficient MPN cells. (**A**) HR activity in in 32Dcl3 parental cells and isogenic MPL(W515L) cells. Results represent mean % ± SD of GFP+ cells in RFP+ cells. (**B**) Lin-CD34+ HR-deficient MPL(W515L)-positive primary MPN cells (n=1) were treated with IC_{50} of Polθi ART558, PARPi olaparib, and RAD52i 6-hydroxy-DL-dopa and with the indicated combinations. Mean ± SD colony numbers from triplicate experiment. Statistical significance when compared to: $^{\#}$ another group, $^{+}$ control, and * corresponding individual treatments.



Supplementary Figure 5. Targeting Polθ + PARP1 and Polθ + RAD52 induced dual synthetic lethality against HR-deficient leukemia cells. A diagram illustrating the model of dual synthetic lethal interactions after targeting Polθ and PARP1 or Polθ and RAD52 in HR-deficient cells (orange) versus HR-proficient cells (blue).