

MYC overexpression in natural killer cell lymphoma: prognostic and therapeutic implications

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Supplementary Materials and Methods

Cell culture

The cell lines included in this study have been described previously.^{1,2} All cell lines were cultured in RPMI 1640 medium supplemented with 15% Fetal Bovine Serum (FBS), 1% Penicillin-Streptomycin (Thermo Fisher Scientific), and 10ng/mL IL-2 (STEMCELL Technologies, Vancouver, BC, Canada). Primary normal NK cells were isolated from fresh healthy human tonsil using the human NK cell isolation kit (Miltenyi Biotec, Gaithersburg, MD, USA) and cultured in RPMI 1640 supplemented with 20% FBS, and 10 ng/mL IL-2.

Cell viability and cell cycle Assays

Cell viability was measured by using the PrestoBlue™ Cell Viability Reagent (Thermo Fisher Scientific Waltham, MA, USA). Relative fluorescence units were detected by Infinite M200 Pro plate reader (Tecan, Männedorf, Switzerland). To measure cell apoptosis, treated cells were stained by using FITC Annexin V Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) and detected by flow cytometry. For the cell cycle analysis, the experimental cells were fixed by cold 75% ethanol and stained by Propidium iodide (Sigma-Aldrich, St. Louis, MO, USA), followed with flow cytometry detection.

Quantitative real-time PCR

RNA was isolated by RNeasy Mini Kit (Qiagen, Germantown, MD, USA), and reversely transcribed to cDNA by using ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA, USA). The real-time quantitative PCR was performed on Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad) using the DyNAmo Flash SYBR Green qPCR Kit (Thermo Fisher Scientific, Waltham, MA, USA). Relative expression fold change was calculated by $2^{-\Delta\Delta CT}$ method. GAPDH was used as the housekeeping gene. PCR primers used were listed in Supplementary Table S1.

Western blot (WB)

The protein was isolated by using M-PER™ Mammalian Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA) supplied with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific, Waltham, MA, USA). Isolated protein was denatured for 10 mins at 70°C in NuPAGE LDS Sample Buffer with reducing buffer (Thermo Fisher Scientific, Waltham, MA, USA). Protein electrophoresis was performed using Bolt 4~12% Bis-Tris Plus Gels and MES SDS running buffer (Thermo Fisher Scientific, Waltham, MA, USA). Proteins were then transferred onto nitrocellulose membrane, blocked with Odyssey TBS Blocking Buffer (LI-COR, Lincoln, NE, USA), and incubated with primary antibody overnight at 4°C. The membrane was washed and incubated with the secondary antibody for 1h at room temperature. After washing, the membrane was scanned on the Odyssey CLx imager (LI-COR). Protein

was quantified based on band intensity using the Image Studio software (LI-COR). Protein expression change was evaluated by normalizing with β -actin or target protein input. The primary antibodies used for immunoblotting are listed in Supplementary Table S2.

Double thymidine block

In cell lines with fresh culture medium, thymidine (Sigma-Aldrich, St. Louis, MO, USA) was added at a final concentration of 2mM and incubated at 37 °C for 18h. Then, the cells were washed with dPBS and cultured with fresh medium at 37 °C for 9h followed by the second round of thymidine treatment at 2mM for another 18h. The cells were then subjected to downstream examination.

Immunohistochemistry (IHC)

Sections from individual formalin-fixed, paraffin-embedded (FFPE) tissues or tissue micro-arrays (TMAs) were stained automatically by BOND-MAX Autostainer (Leica Biosystems, Wetzlar, Germany) according to the manufacturer's protocol. The primary antibodies used for IHC are listed in Supplementary Table S2. Photographs were taken using the Leica Aperio CS2 scanning system with 40X magnification.

Fluorescence in situ hybridization (FISH)

FISH was performed on FFPE tissues for 60 ENKTL cases. The MYC Dual Color Break Apart Rearrangement Probe and the IGH/MYC/CEP 8 Tri-Color DF FISH Probe (Vysis; Abbott Molecular, Desplaines, IL, USA) were used to interrogate the MYC locus at chromosome 8q24. and the presence of MYC translocation/amplification according to the manufacturer's protocol in the FFPE specimens, respectively.

Briefly, 3–4 μ m-thick FFPE tissue sections were cut and incubated at 56°C for 3 hours. After deparaffinizing and dehydrating the sections, they were incubated in 2x saline sodium citrate buffer (2x SSC, pH 7.0) at 75°C for 20 minutes and were then digested with proteinase K (0.2 mg/mL) at 37°C for 20 minutes. Probe sets were applied onto the tissue sections on each slide followed by denaturation at 80°C for 5 minutes. Probes were then hybridized overnight at 37°C using the ThermoBrite system (Vysis). Nuclei were counterstained with 4,6-di-amidino-2-phenylindole (DAPI, Vysis) and FISH signals were assessed using an Olympus BX61 microscope (Olympus, Tokyo, Japan). Hybridization signals were assessed in 200 interphase nuclei, with an established cutoff of 15% for *MYC* rearrangement of the locus, and copy numbers >5 or the ratio of green to red signal > 2 for *MYC* amplification. Images were acquired using the BioView Automated Imaging Analysis System (BioView, Rehovot, Israel).

Statistics and data analysis

The Overall Survival (OS) and Progression-Free Survival (PFS) curves were analyzed by the Kaplan-Meier method. For the RNA sequencing, FPKM was used to estimate gene expression levels, and DESeq2 method³ was used to analyze the differential

gene expression with the screening threshold of $\log_2(\text{FoldChange}) \geq 1$ and $\text{padj} \leq 0.05$. Gene expression and pathway enrichment analysis was based on the GSEA/MSigDB database. The Data from functional and animal studies were analyzed by using GraphPad Prism 9 software. Data shown with the mean \pm SD are from at least two independent experiments. Unpaired t-test was used to compare data from two independent groups. One-way analysis of variance (ANOVA) was used to compare data from three or more independent groups. *P*-values of less than 0.05 were considered significant.

Reference

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Supplementary Table S1. List of oligos used in this study

qRT-PCR primers		Forward	Reverse
MYC-1		GGTGCTCCATGAGGAGACA	CCTGCCTCTTTTCCACAGAA
MYC-2		GGACCCGCTTCTCTGAAAGG	TAACGTTGAGGGGCATCGTC
GAPDH		CCACTCCTCCACCTTTGAC	ACCCTGTTGCTGTAGCCA
siRNA			
MYC-S1	MYC-1	rArUrCrArUrUrGrArGrCrCrArArArUrCrUrUrArArArAAA rUrUrUrUrUrUrArArGrArUrUrUrGrGrCrUrCrArArUrGrArUrArU	
	MYC-2	rGrGrArArCrGrArGrCrUrArArArArCrGrGrArGrCrUrUrUTT rArArArArArGrCrUrCrCrGrUrUrUrArGrCrUrCrGrUrUrCrCrUrC	
MYC-S2	MYC-3	rCrGrArCrGrArGrArCrCrUrUrCrArUrCrArArArArCrATC rGrArUrGrUrUrUrUrGrArUrGrArArGrGrUrCrUrCrGrUrCrGrUrC	
	MYC-4	rArGrGrArArArArCrGrArUrUrCrCrUrUrCrUrArArCrArGAA rUrUrCrUrGrUrUrArGrArArGrGrArArUrCrGrUrUrUrUrCrCrUrUrA	
MYC-S3	MYC-5	rUrArUrArUrCrArUrUrGrArGrCrCrArArArUrCrUrUrArAAA rUrUrUrUrArArGrArUrUrUrGrGrCrUrCrArArUrGrArUrArUrUrU	
	MYC-6	rGrArCrUrGrArArArGrArUrUrUrArGrCrCrArUrArArUrGTA rUrArCrArUrUrArUrGrGrCrUrArArArUrCrUrUrUrCrArGrUrCrUrC	
Rb-1		rGrUrArCrCrArArArGrUrUrGrArUrArArUrGrCrUrArUrGTC rGrArCrArUrArGrCrArUrUrArUrCrArArCrUrUrUrGrGrUrArCrUrG	
Rb-2		rGrGrArUrUrArUrUrGrArUrArGrUrArCrUrCrUrUrGrGrUTT rArArArCrCrArArGrArGrUrArCrUrArUrCrArArUrArArUrCrCrUrC	

Supplementary Table S2. List of antibodies used for WB and IHC

Target protein	Manufacturer	Clone	Application
MYC	Abcam	Y69	WB/IHC
HK2	Abcam	EPR20839	WB
CDK4	Cell Signaling Technology	D9G3E	WB
Rb	Thermo Fisher Scientific	1F8	WB
p-Rb (S780)	Abcam	EPR182(N)	WB
p-Rb (S795)	Cell Signaling Technology	Polyclonal	WB
p-Rb (S807/811)	Cell Signaling Technology	D20B12	WB
β -Actin	Santa Cruz	C4	WB
Ki-67	Dako	MIB-1	IHC
CD3e	Thermo Fisher Scientific	SP7	IHC
CD56	Cell Signaling Technology	E7X9M	IHC

Supplementary Table S3. Correlation between MYC expression and major clinical features in this cohort of ENKTL cases (N=111)

Characteristics	MYC		p-value
	Low level n (%)	High level n (%)	
Patients	28 (25.2)	83 (74.8)	
Age	≤60	26 (25.2)	1.000
	>60	2 (25.0)	
Gender	Male	16 (21.6)	0.216
	Female	12 (32.4)	
ECOG performance	<2	26 (26.3)	0.727
	≥2	2 (16.7)	
Ann Arbor staging	I /II	24 (28.2)	0.187
	III/IV	4 (15.4)	
B symptoms	Absence	13 (22.8)	0.547
	Presence	15 (27.8)	
Primary tumor site	UAT	25 (27.5)	0.245
	Non-UAT	3 (15.0)	
PTI	No	17 (26.6)	0.705
	Yes	11 (23.4)	
Regional LN involvement	No	16 (25.8)	1.000
	Yes	12 (24.5)	
Distant organ metastasis	No	25 (27.2)	0.392
	Yes	3 (15.8)	
LDH	Normal	24 (28.9)	0.123
	Elevated	4 (14.3)	
Epstein-Barr virus DNA	Undetectable	8 (29.6)	0.545
	detectable	20 (23.8)	
PINK-E score	<2	23 (27.1)	0.421
	≥2	5 (19.2)	
Treatment mode	Chemotherapy	7 (16.7)	0.105
	CMT	21 (30.4)	
Chemotherapy regimens	Peg/Asp -based	22 (25.6)	0.986
	Anthracyclines-based	5 (23.8)	
	Others	1 (25.0)	
Treatment response	CR	22 (26.2)	0.909
	Non-CR	5 (21.7)	
	Unknown	1 (25.0)	

Abbreviations: Asp, Asparaginase; CMT, Combinational modality treatment; ECOG, Eastern Cooperative Oncology Group; LDH, Lactate dehydrogenase; LN, Lymph node; PTI, Primary tumor invasion; Peg,

Pegaspargase; PINK-E, Prognostic index for natural killer cell lymphoma-EBV; UAT, Upper aerodigestive tract.

Supplementary Table S4. Multivariate analysis of OS by the Cox proportional hazards model

Prognostic factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age>60	—	—	0.113	—	—	—
Stage III/IV	1.999	1.066-3.748	0.028	—	—	—
ECOG performance status ≥ 2	2.741	1.276-5.886	0.007	3.032	1.404-6.546	0.003
Elevated LDH	—	—	0.093	—	—	—
Detectable Epstein-Barr virus DNA	—	—	0.217	—	—	—
Primary tumor invasion	—	—	0.105	—	—	—
Non-UAT	—	—	0.442	—	—	—
Regional LN involvement	—	—	0.247	—	—	—
Distant organ metastasis	2.106	1.071-4.144	0.027	—	—	—
Ki67 $\geq 65\%$	2.337	1.296-4.212	0.004	2.457	1.359-4.441	0.005
c-Myc $\geq 20\%$	2.110	0.985-4.523	0.049	—	—	—

Non-UAT, non-upper aerodigestive tract; LN, Lymph node.

Supplementary Table S5. Multivariate analysis of PFS by the Cox proportional hazards model

Prognostic factors	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age>60	1.10	0.40-3.05	0.847	-	-	-
Stage III/IV	2.49	1.44-4.32	0.001	1.92	0.74-4.96	0.180
ECOG performance status ≥ 2	1.87	0.89-3.94	0.010	1.83	0.85-3.94	0.125
Elevated LDH	1.63	0.93-2.87	0.089	1.19	0.67-2.14	0.533
Detectable Epstein-Barr virus DNA	1.40	0.76-2.59	0.281	-	-	-
Primary tumor invasion	1.03	0.61-1.73	0.903	-	-	-
Non-UAT	1.40	0.76-2.6	0.280	-	-	-
Regional LN involvement	1.25	0.75-2.07	0.396	-	-	-
Distant organ metastasis	2.38	1.30-4.35	0.005	0.98	0.34-2.82	0.970
Ki67 $\geq 65\%$	2.28	1.36-3.82	0.002	1.49	0.81-2.75	0.201
c-Myc $\geq 20\%$	2.72	1.33-5.55	0.006	1.86	0.81-4.26	0.141

Non-UAT, non-upper aerodigestive tract; LN, Lymph node.

Supplementary Table S6. Commonly altered genes in primary case and cell line analysis

RNA-seq data Log2 transformed

Gene	NK24_C1	NK24_C2	NK24_M1	NK24_M2	NK48_C1	NK48_C2	NK48_M1	NK48_M2	IM24_C1	IM24_C2	IM24_M1	IM24_M2	IM48_C1	IM48_C2	IM48_M1	IM48_M2
HPDL	10.9993	10.8979	8.84786	9.33826	10.4654	10.1984	8.54135	9.30939	9.89395	9.66614	7.37461	6.28251	10.2985	10.3253	8.33512	8.39229
TRAP1	11.8349	11.7087	9.9496	10.239	11.654	11.3752	9.95449	10.2545	11.645	11.6569	9.63602	9.16097	12.3642	12.2898	10.6898	10.2265
RNASEH1	8.74483	8.59986	6.9649	6.92813	8.58825	7.8394	6.52609	6.85873	8.12969	7.77138	6.57115	5.73231	8.46367	8.16231	6.85064	6.30593
TTLL12	11.7561	11.5909	9.69247	9.97253	11.247	10.9011	9.79646	9.98939	12.62	12.5487	10.8746	10.5043	12.9141	12.8233	11.779	11.537
HSPD1	14.422	14.5989	12.716	13.1674	14.3198	13.9299	12.8004	12.9966	14.7191	14.7038	12.8646	11.8951	14.9769	14.9468	13.8545	13.2439
IFRD2	11.2174	11.0053	9.46465	9.66432	10.911	10.4428	9.60993	9.76722	11.9305	11.7051	9.84041	9.07724	11.9242	11.7861	10.9153	10.6128
RUVBL1	12.0931	12.0919	10.5769	10.7796	11.9422	11.5475	10.5807	10.8222	11.7178	11.5537	10.117	9.42679	12.0236	11.7976	10.6749	10.4131
HK2	11.7814	11.7493	9.83639	10.4854	11.4728	11.4428	9.91548	10.3028	11.4763	11.5131	9.71667	10.1148	11.6114	11.8959	10.7611	10.3645
SLC19A1	10.8719	10.8011	8.71492	9.22156	10.4388	9.6994	9.04564	9.33338	11.3445	11.3809	9.67136	9.11971	11.4418	11.2387	10.6351	10.4336
PRMT3	10.444	10.4312	9.15139	9.34121	10.2838	10.1173	9.19067	9.18546	10.0391	10.0128	8.39012	7.63231	10.1965	10.1888	9.15392	8.67577
FBL	12.8742	12.8221	11.0829	11.3486	12.5785	12.1713	11.2342	11.467	12.4019	12.3767	11.3169	11.1723	13.1034	12.9408	11.687	11.3879
FAM216A	9.89936	9.80913	8.23339	8.61403	9.71501	9.32943	8.24762	8.8707	10.2137	10.2781	8.62786	8.15858	10.4933	10.4735	9.45314	9.07807
EBPL	10.6636	10.6917	8.90024	8.96299	10.3057	9.72025	8.73968	9.0525	9.74671	9.50545	8.6081	7.99104	10.1172	9.97981	8.97727	8.6902
WDR74	10.3038	10.2956	8.89533	9.01928	9.877	9.27843	8.58988	8.72992	10.2175	10.1291	8.77101	8.3301	10.5548	10.2654	9.1323	9.04477
NOLC1	13.1524	13.2206	11.685	12.0188	12.9971	12.5884	11.713	12.0376	13.2399	13.3498	11.5077	10.6184	13.3655	13.3106	12.6004	12.0612
DPH5	9.77808	9.95923	8.50547	8.982	9.69367	9.61799	8.46735	8.79419	9.2111	9.26146	7.79742	7.23784	9.82555	9.5229	8.4444	8.1787
PHB	11.9133	11.8868	10.3347	10.5569	11.6384	11.0842	10.2499	10.4987	11.6009	11.4923	10.2526	9.8137	11.8839	11.7727	10.901	10.5149
MRPL4	11.0888	10.9054	9.80402	9.82264	10.7398	10.2763	9.66498	9.71671	10.8121	10.7291	9.16966	8.99642	11.3072	11.1433	10.1117	9.91105
TUFM	12.8595	12.8387	11.6366	11.7049	12.5328	12.2646	11.5333	11.7716	12.46	12.2965	10.9802	10.6537	12.9433	12.8571	11.6104	11.4115
PRMT1	13.0636	13.1134	11.4667	11.7812	12.7635	12.2994	11.5723	11.7303	12.4448	12.4106	11.1476	11.1859	12.8199	12.7235	11.7155	11.4805
PAICS	13.1813	13.2289	11.6932	12.2002	13.0356	12.7678	11.9438	12.031	13.5156	13.5048	11.8524	11.0289	13.396	13.3379	12.7617	12.3009
RCC1	12.0126	11.9906	10.4933	10.8245	11.7596	11.3258	10.5761	10.8237	12.1538	12.1931	10.8148	10.4047	12.4439	12.2563	11.5321	11.3773
IARS	12.2476	12.3135	11.092	11.4144	12.2303	12.1203	11.2141	11.3247	12.7785	12.6901	11.1292	10.5769	12.9214	12.8931	12.2769	11.6762
PFAS	10.7977	10.5278	9.83639	9.62616	10.5027	10.4086	9.58437	9.91906	10.8397	10.6336	9.19417	8.46023	11.2746	11.0141	10.1752	9.80899
HSP90AB	15.9053	15.9839	14.5715	14.8587	15.7169	15.4402	14.7298	14.8867	15.4669	15.479	14.0179	13.2984	15.6854	15.7161	14.9795	14.5015
MRPL3	12.2825	12.3729	11.0791	11.2918	11.9549	11.3625	10.914	10.9846	11.8626	11.8261	10.5385	10.1197	12.0486	11.9826	11.2309	10.794
RPL22	10.8556	10.9599	9.57923	9.82242	10.5016	10.1413	9.45934	9.76402	10.7432	10.8045	9.70276	9.19642	10.8207	10.7983	9.81038	9.45066
EEF2KMT	8.69	8.55165	7.64063	7.79689	8.59664	8.04155	7.56395	7.57389	7.9082	7.98651	6.65058	5.65288	8.0334	7.80545	6.76896	6.51488
RUVBL2	12.0133	11.8683	10.7809	10.8974	11.8813	11.6807	10.8442	10.9859	11.4088	11.2902	10.4717	10.1405	12.1284	12.1416	10.9334	10.7671
LIAS	8.93074	9.06483	8.23339	7.8392	8.65403	8.48185	7.86631	7.74625	9.46346	9.35151	7.79159	7.68651	9.58074	9.12852	8.48334	8.13718
XPOT	11.5684	11.466	10.5708	10.5339	11.4683	11.3746	10.4613	10.4852	12.5989	12.5084	11.2913	10.6706	12.6888	12.6996	12.093	11.626
NOB1	10.5068	10.5965	9.19995	9.40457	10.2201	9.70585	9.16379	9.32688	10.2162	10.2102	8.98533	8.78914	10.4637	10.3761	9.63896	9.35203
DFFA	12.1315	12.0398	10.9637	11.023	12.0511	11.6505	11.1681	11.1218	11.6586	11.6019	10.4131	10.0335	11.8207	11.7158	11.0403	10.6548
POLD2	11.5063	11.2919	10.1667	10.3324	11.1385	10.8453	10.2523	10.251	11.7507	11.6316	10.7539	10.2115	12.083	12.0392	11.1646	10.9164
YBX1	15.0723	15.1732	13.7994	14.1012	14.8902	14.5656	13.9136	14.0702	15.1286	15.0726	14.1658	13.7935	15.6702	15.6038	14.7027	14.4985
CCT7	13.7444	13.7329	12.6239	12.7405	13.4605	13.0605	12.4741	12.6586	13.6589	13.6589	12.4839	12.1649	13.9302	13.7668	12.9914	12.8378
SRM	13.3706	13.368	12.128	12.1024	12.6755	12.0038	11.8154	11.5518	13.4241	13.3577	12.3021	11.9525	13.5537	13.0781	12.6926	12.4011
PRMT5	9.94837	9.83526	8.67826	8.53386	9.57982	8.82762	8.38512	8.52169	10.1525	10.0326	8.98533	8.91931	10.41	10.1745	9.58827	9.35203
EIF3F	12.7087	12.7803	11.6132	11.9777	12.5809	12.3279	11.4936	11.7515	12.4454	12.3705	11.2711	10.9228	12.5251	12.4381	11.844	11.4309
CAD	12.0505	11.8764	11.127	11.1544	11.8571	11.6655	11.125	11.0293	12.3685	12.2615	11.0244	10.4198	12.5217	12.4568	11.8197	11.6
DHX33	11.3902	11.5225	10.268	10.6006	11.1906	10.5869	10.3224	10.3739	11.7884	11.9449	10.431	9.91931	11.7723	11.614	11.2511	10.8909
LRPPRC	11.8568	12.0174	10.9841	11.2994	11.7855	11.5934	11.0891	11.1849	12.8121	12.9836	11.4575	11.0677	12.9118	12.8986	12.2306	11.834
MDH2	12.9998	12.9576	11.989	12.0453	12.8224	12.7357	11.9659	12.1457	12.7221	12.5647	11.6389	11.2441	13.0264	13.0819	12.2486	12.1022
NPM1	15.6251	15.7651	14.3728	14.787	15.3295	15.0254	14.2499	14.5578	15.4326	15.4817	14.3599	14.0236	15.4873	15.2727	14.9281	14.7131
CCDC51	9.60548	9.49633	8.32747	8.6525	9.20785	8.85108	8.15141	8.41536	8.96684	9.16543	8.20517	7.92496	9.36973	9.32231	8.19098	8.48623
SERBP1	14.2652	14.3543	13.2217	13.5519	13.9674	13.8163	13.2284	13.3979	14.2313	14.3393	13.1534	12.7673	14.3932	14.3234	13.6885	13.3727
MCCC2	11.4929	11.6648	10.9742	11.2143	11.47	11.3665	10.7829	10.9554	11.2754	11.3412	9.92804	9.4872	11.4913	11.5491	10.6972	10.2523
MRPS25	10.3923	10.1792	9.17182	9.42138	10.1189	9.81575	9.24529	9.05513	10.4199	10.5206	9.52622	9.32797	10.7914	10.6936	10.0425	9.83194
CCDC124	11.8486	11.7516	10.6711	10.5757	11.247	10.8511	10.3058	10.4577	10.7755	10.6768	10.0343	9.81218	11.3464	11.2875	10.3539	10.4272
SSBP1	11.749	11.7966	10.5884	10.7709	11.2497	10.6962	10.3235	10.401	11.302	11.2531	10.6417	10.4128	11.7505	11.547	10.6148	10.5889
CCT4	12.7187	12.7575	11.8966	12.1295	12.4796	12.2004	11.6619	11.8234	12.7247	12.7181	11.4898	11.1953	12.6994	12.6358	11.9983	11.7305
PDHA1	11.445	11.4959	10.6732	10.8055	11.4046	11.1875	10.6045	10.7831	11.2414	11.2162	10.3047	9.89218	11.65	11.7097	10.8556	10.609
CCT2	12.9388	13.0483	11.9643	12.2512	12.6442	12.2348	11.9789	11.9115	13.0981	13.1814	12.0253	11.6668	13.0702	12.9622	12.5357	12.2169
CSE1L	12.42	12.6182	11.5561	12.0059	12.3566	12.279	11.7623	11.8125	12.5748	12.5426	11.3877	11.0523	12.6566	12.5365	12.0551	11.6924
MYC	13.4584	13.3451	12.7174	12.714	12.6924	12.5722	11.8446	12.3535	13.004	12.9069	11.8021	11.3554	13.1168	13.1021	12.3973	12.3597
ATIC	12.445	12.3055	11.3719	11.6376	12.1739	11.9392	11.3665	11.6194	12.1495	12.0337	11.0563	10.2559	12.0891	12.1105	11.5917	11.4022
MRPL16	10.4409	10.3858	9.34197	9.59687	10.1058	9.95488	9.44723	9.57389	10.4429	10.3851	9.75162	9.41681	10.6469	10.6046	9.74423	9.62973
LARP1	13.8332	13.8217	12.8904	13.0915	13.6622	13.3471	12.7576	12.9373	13.6894	13.695	12.7482	12.3892	13.8927	13.7999	13.475	13.2328
SLC25A5	13.0923	13.0578	12.2701	12.3434	12.9062	12.6797	12.1852	12.3299	13.0931	12.9491	12.2542	11.6469	13.4072	13.363	12.7991	12.5576
CDK4	11.294	11.2309	10.4762	10.4639	10.											

Supplementary Figure Legends:

Supplementary Figure S1. Genetic examination of MYC abnormality in ENKTL. FISH was performed by using MYC break-apart and fusion probes respectively in 60 cases. (A) representative figures for the fusion probe. (B) Left, a representative figure showing MYC gain (MYC-CN/Chr 8-CN <2); Right, a summary of the FISH results.

Supplementary Figure S2. Survival outcomes of patients receiving pegaspargase/asparaginase-based treatment. The patients were grouped by MYC expression status, and the PFS (A) and OS (B) curves are shown.

Supplementary Figure S3. MYC KD in NK malignancy cells with low and intermediate MYC Expression. YT, NK-YS, and SNK-6 cells were knocked down with MYC by siRNA mixture S1. (A) MYC protein levels were measured 24 hours after siRNA transfection. (B) Cell viability was measured 72 hours after transfection.

Supplementary Figure S4. Transcriptome alteration after MYC KD in NK malignancy cells. RNA-seq was performed to measure the transcriptome alteration after 24h of MYC KD in NK-92 and IMC-1 cells. (A) The volcano plots showing the relative gene expression of MYC KD to control cells. Those with expression levels ≥ 2 -fold were subject to further analysis. (B) The significantly altered genes in NK-92 and IMC-1 were analyzed by GSEA, respectively, and the enrichment of the "MYC_targets" signature is shown. (C-D) The commonly down-regulated (C) and up-regulated (D) genes between NK-92 and IMC-1 cells were examined with molecular signature enrichment. The Venn diagrams show the relationship of the altered genes between these two cells, and the Hallmark gene sets were used for the signature analysis.

Supplementary Figure S5. Transcriptome alteration after 48h of MYC knockdown in NK-92 and IMC-1 cells. (A) The volcano plots showing the relative gene expression of MYC knockdown to control cells. Those with expression levels ≥ 2 -fold were subject to further analysis. (B) The commonly down-regulated and up-regulated genes between NK-92 and IMC-1 cells were examined with molecular signature enrichment using the Hallmark gene set. (C) The Venn diagrams showing the common down-regulation and up-regulation between these two cells.

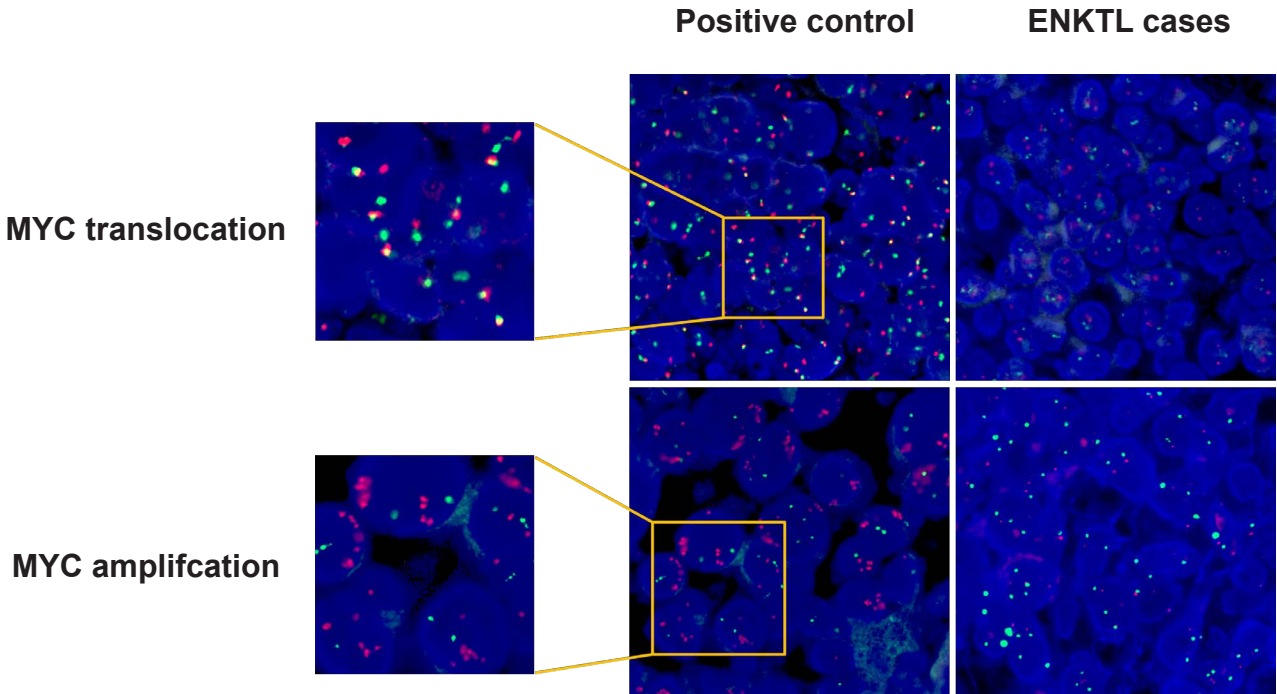
Supplementary Figure S6. CDK4 as a potential therapeutic target in ENKTL with MYC overexpression. (A) MYC-low NK malignancy cell lines YT and NK-YS as well as BL cell lines Namalwa and Raji were knocked down with MYC for 48h and examined with MYC, HK2, and CDK4 by WB. (B) NK malignancy cell lines were treated with increasing doses of benserazide for 72h and examined with cell viability by prestobule assay. The cell lines are ranked by the area under curve (AUC) of the inhibition plot. (C) KHYG-1, NK-92, and IMC-1 cells were treated with palbociclib (1 μ M) and examined with MYC mRNA level by qRT-PCR after 24h and 48h of treatment, respectively. (D) Namalwa and Raji cells were treated with palbociclib and examined with MYC protein level by WB after 24h and 48h of treatment. (E) NK malignancy cells were subjected to double thymidine block and examined with MYC level by WB.

Supplementary Figure S7. Pathological examination for IMC-1 CDX model. (A-B) Spleen and liver involvement of the xenograft tumor cells. (C-F) IHC staining of CD3e, CD56, MYC, and Ki-67 in the liver with tumor involvement.

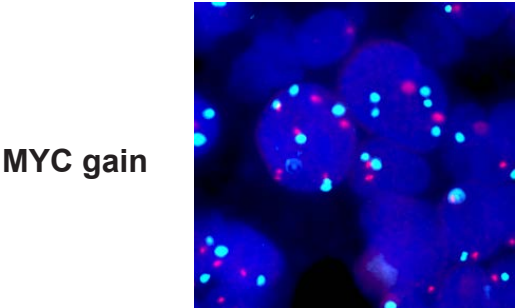
Supplementary Figure S8. Palbociclib treatment in the YT-CDX model. The volume of the subcutaneous tumor was calculated using the formula $V = (W^2 \times L)/2$, and compared between saline control and palbociclib-treated groups.

Supplementary Figure S1

A



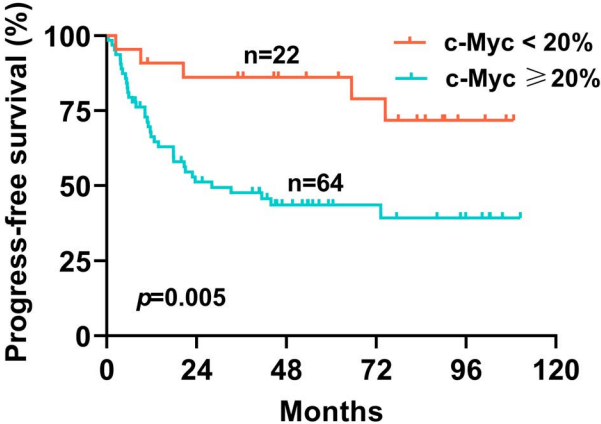
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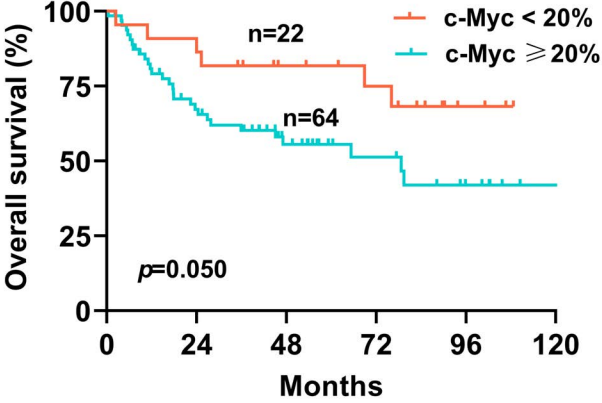
Genetic abnormality	Case number	MYC IHC
MYC translocation	0/60	N/A
MYC amplification	0/60	N/A
MYC gain	3/60	2/3

Supplementary Figure S2

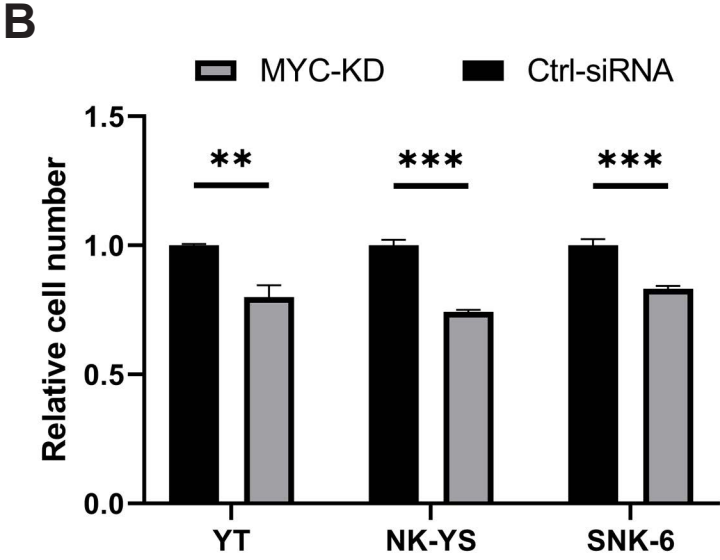
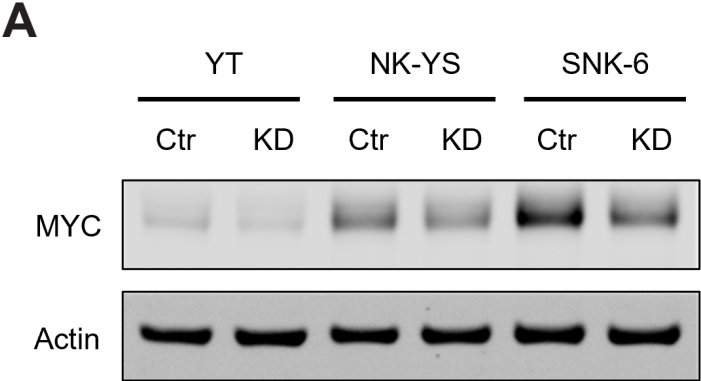
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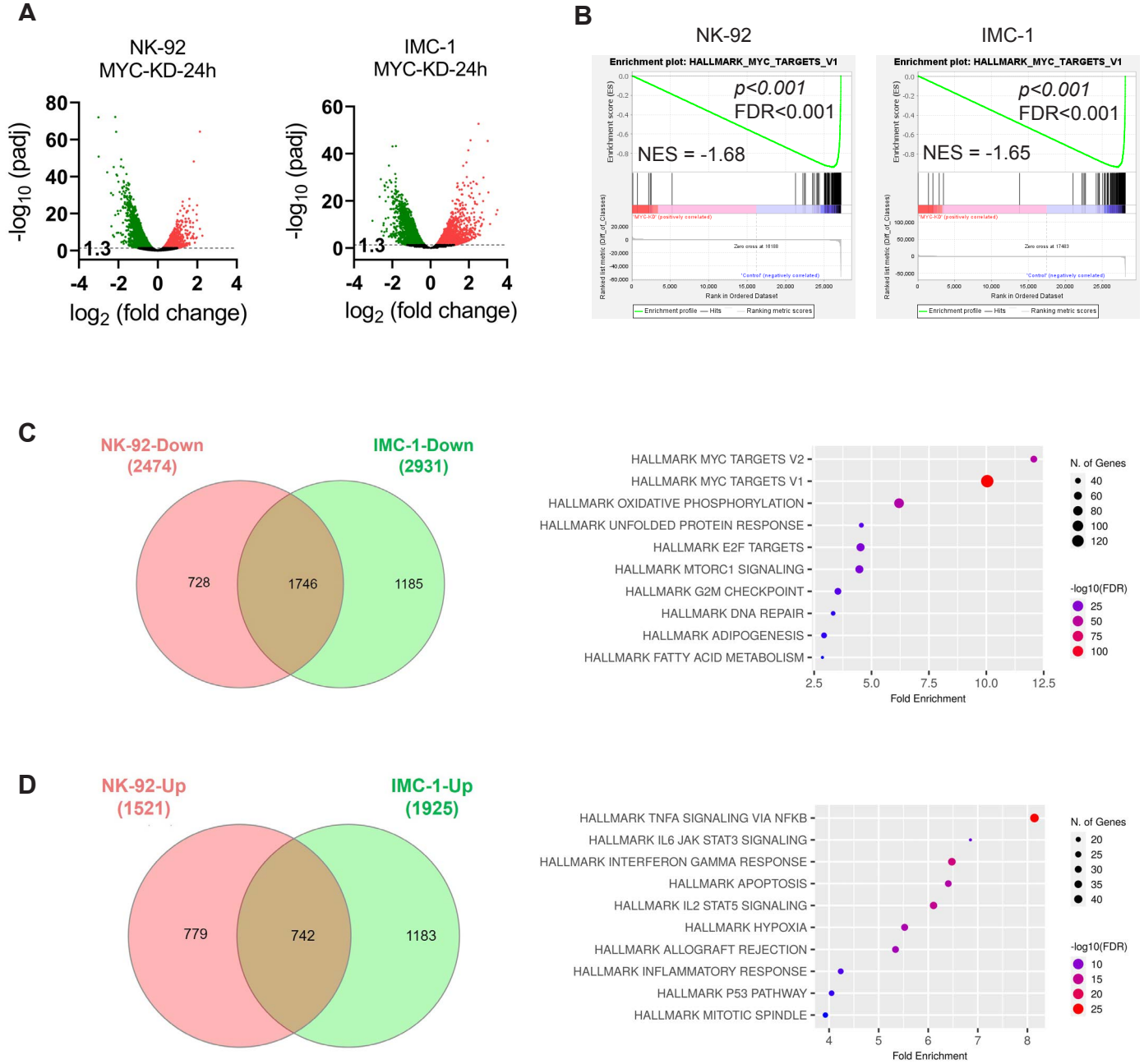
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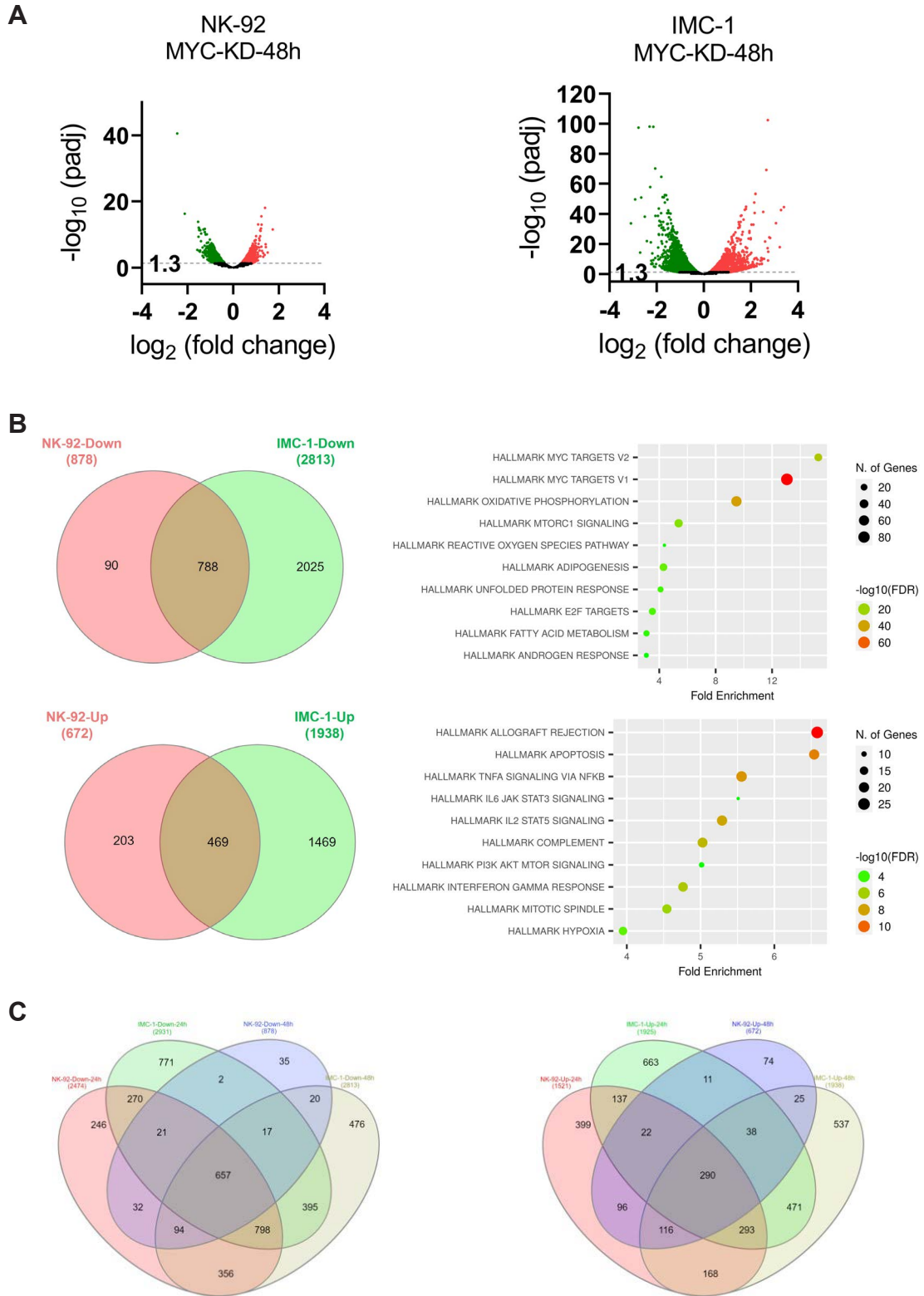
Supplementary Figure S3



Supplementary Figure S4

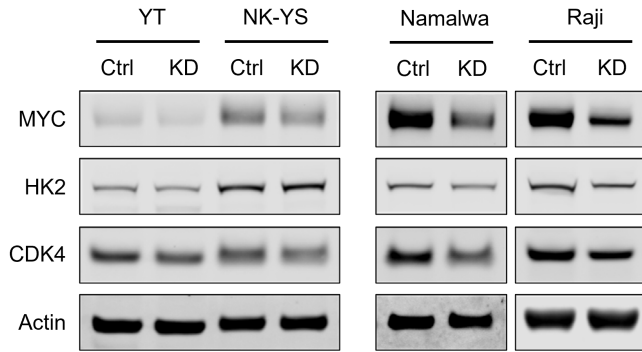


Supplementary Figure S5

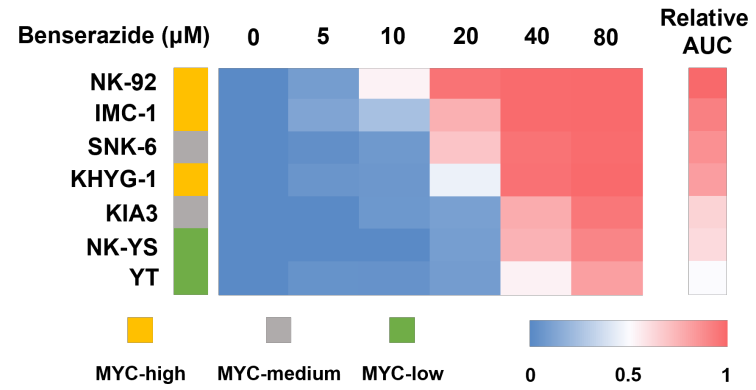


Supplementary Figure S6

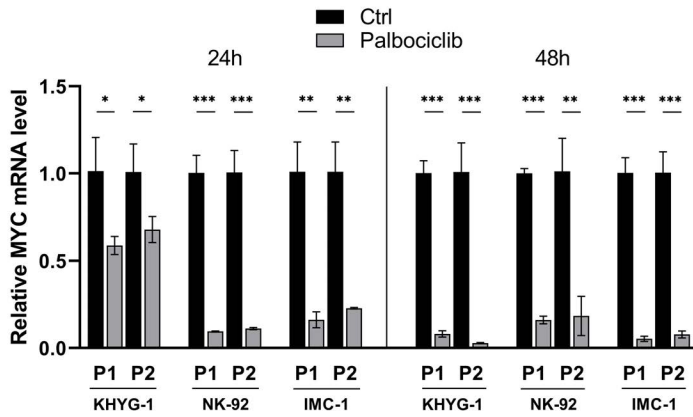
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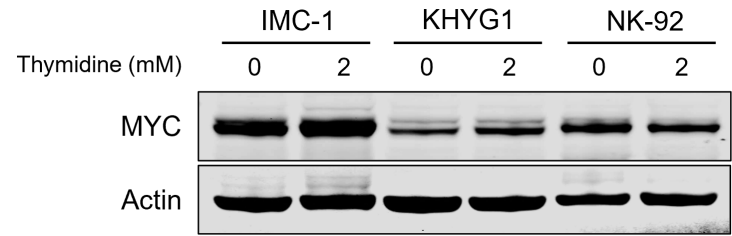
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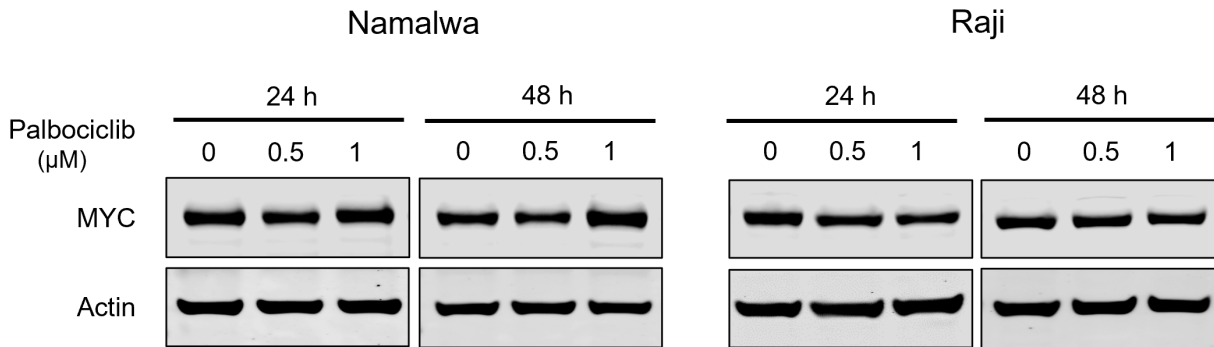
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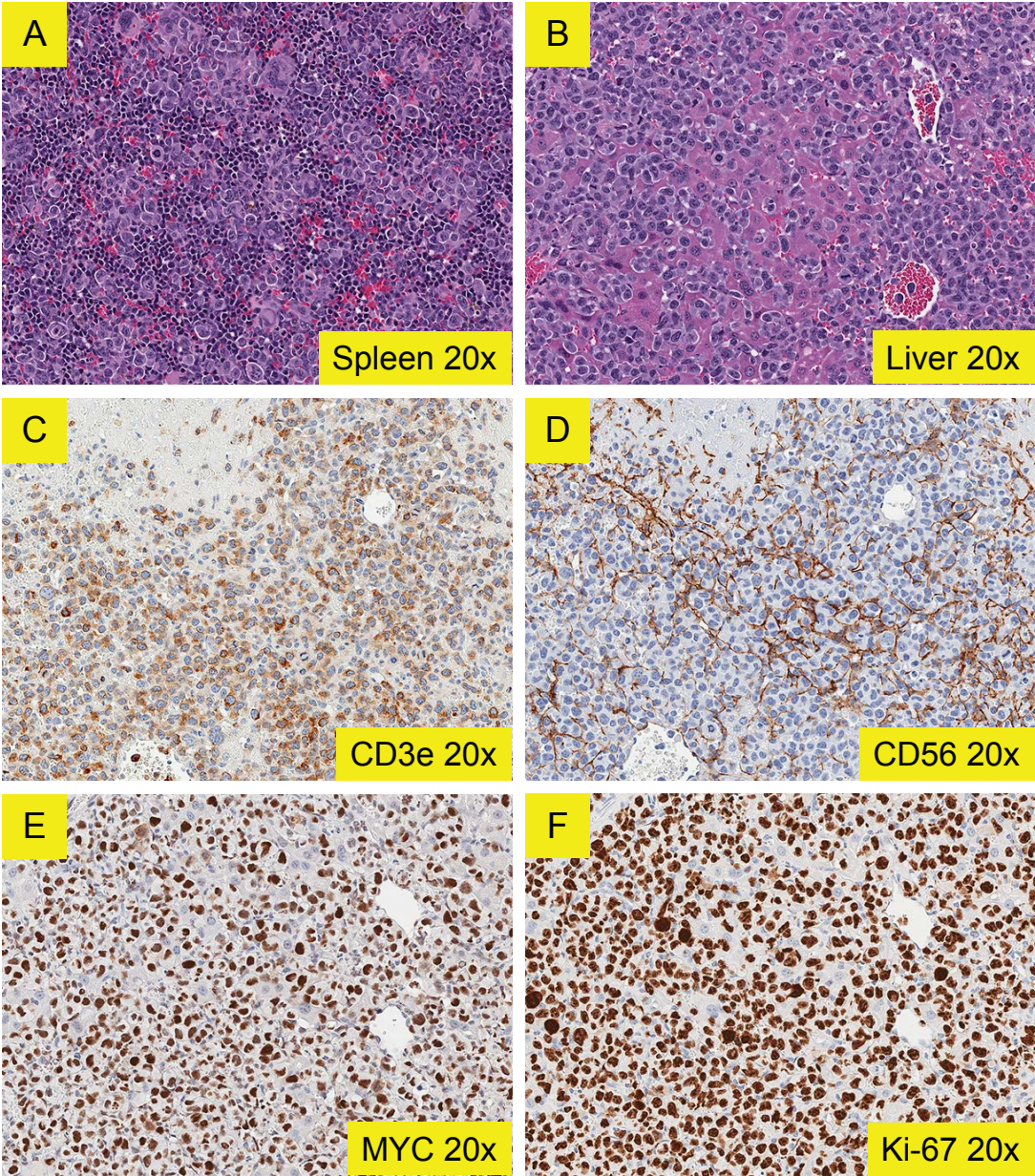
E



D



Supplementary Figure S7



Supplementary Figure S8

