

Identification of PIKfyve kinase as a target in multiple myeloma

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Supplemental Data

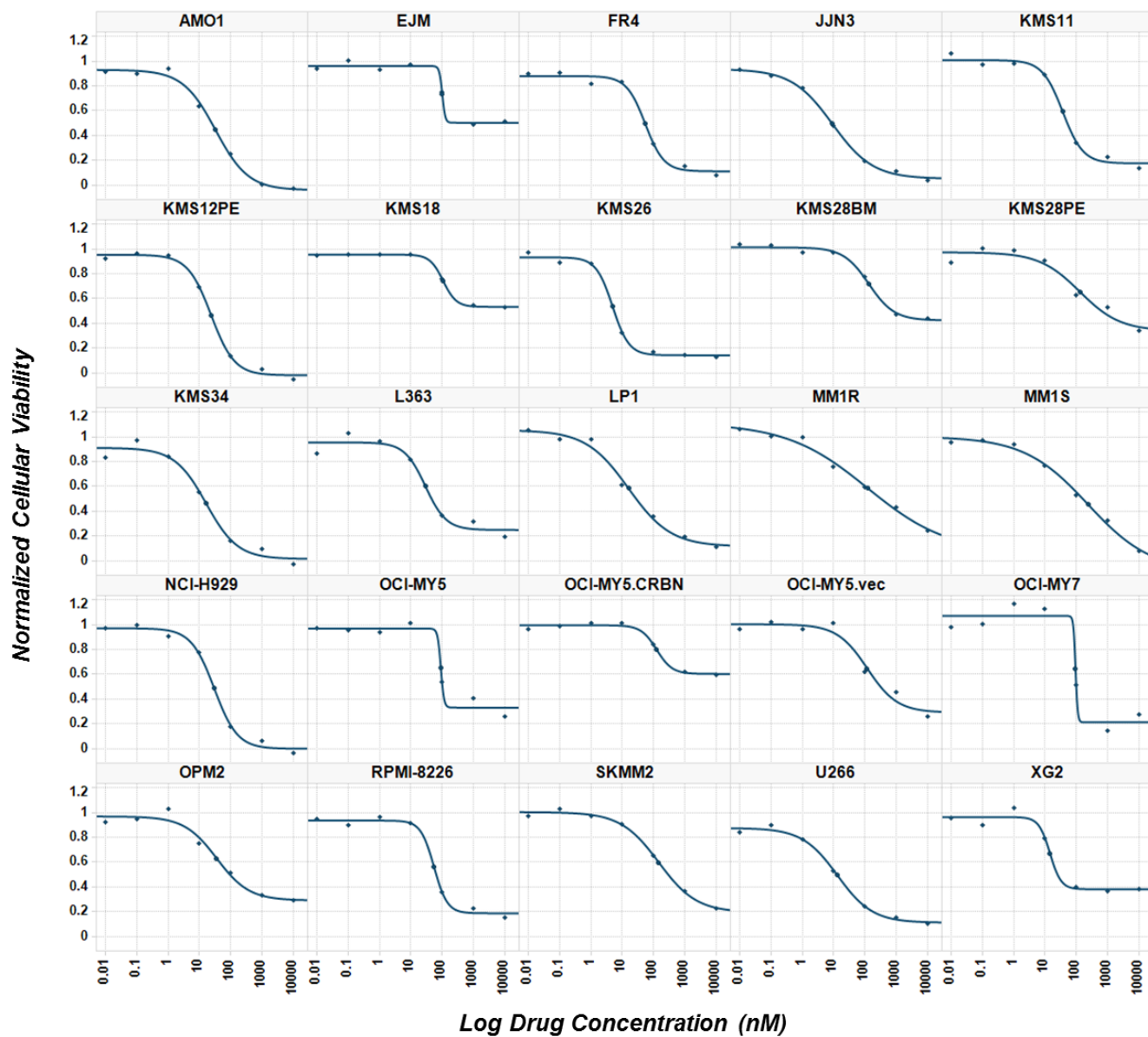


Figure S1. Normalized cellular viability of 25 human myeloma cell lines incubated for 72 hours with a 7-point 10-fold dilution starting at 10 μ M of APY0201.

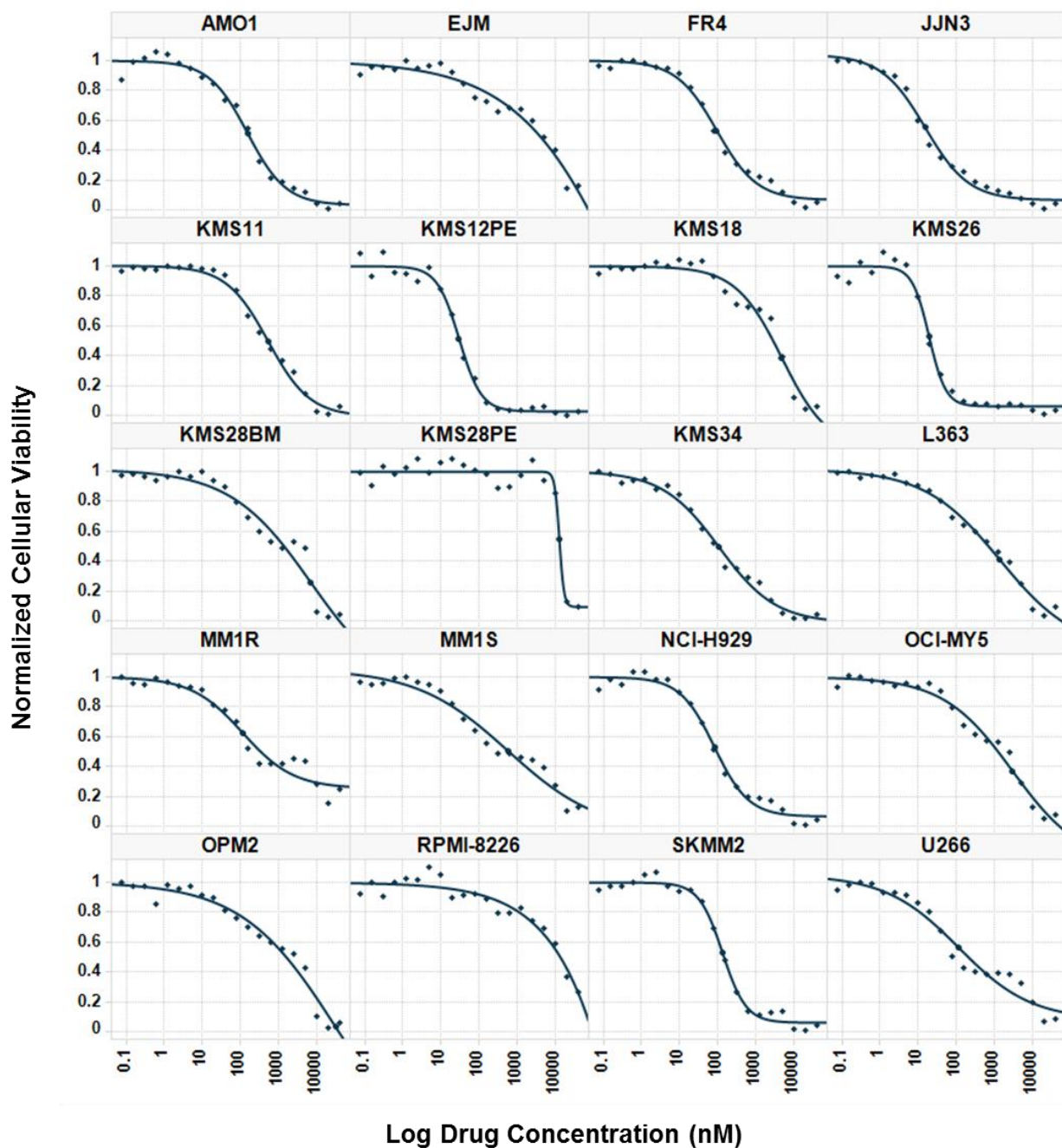


Figure S2. Normalized cellular viability of 20 human myeloma cell lines incubated for 72 hours with a 20-point 2-fold dilution starting at 40 μ M of APY0201.

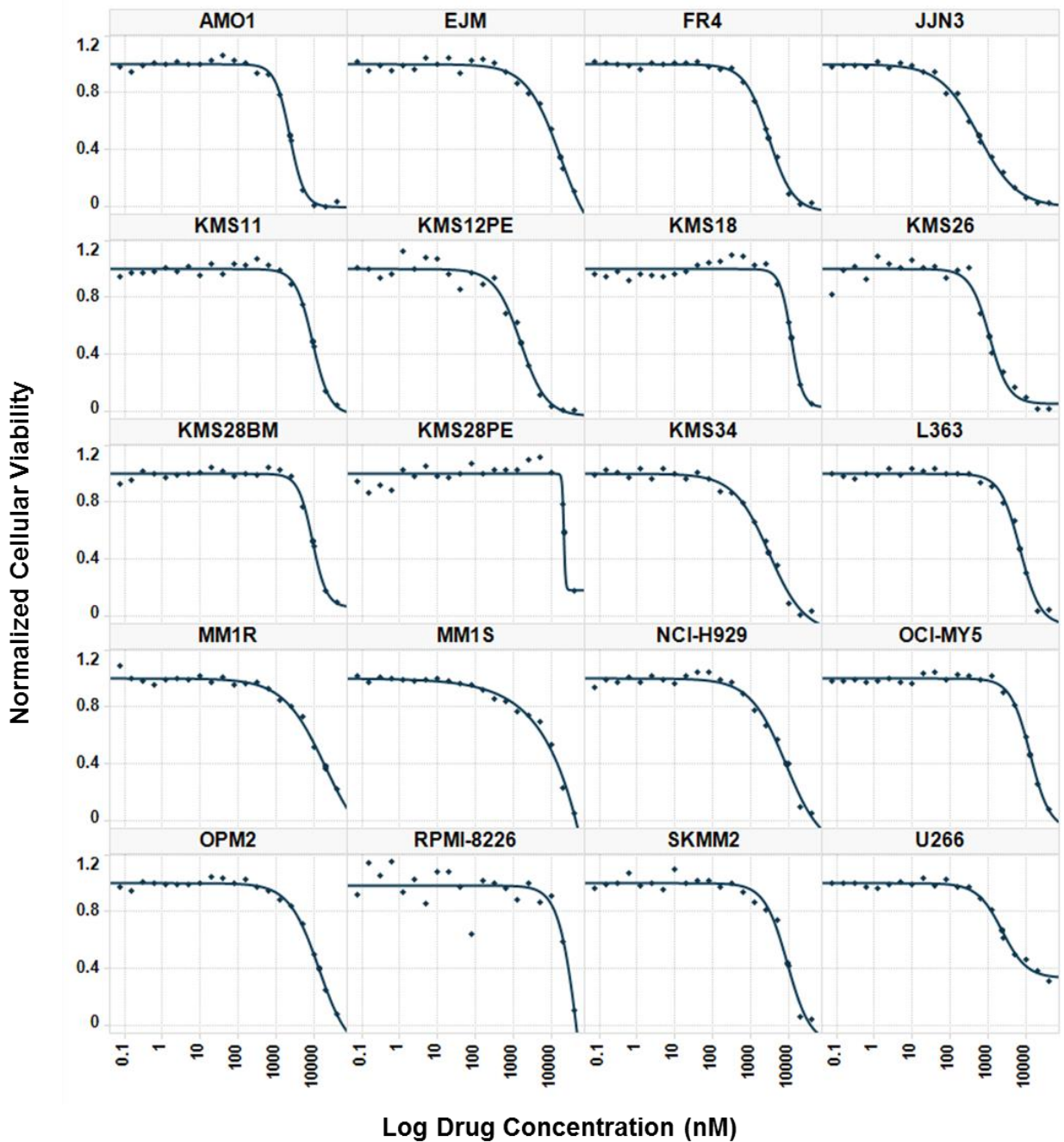


Figure S3. Normalized cellular viability of 20 human myeloma cell lines incubated for 72 hours with a 20-point 2-fold dilution starting at 40 μ M of apilimod.

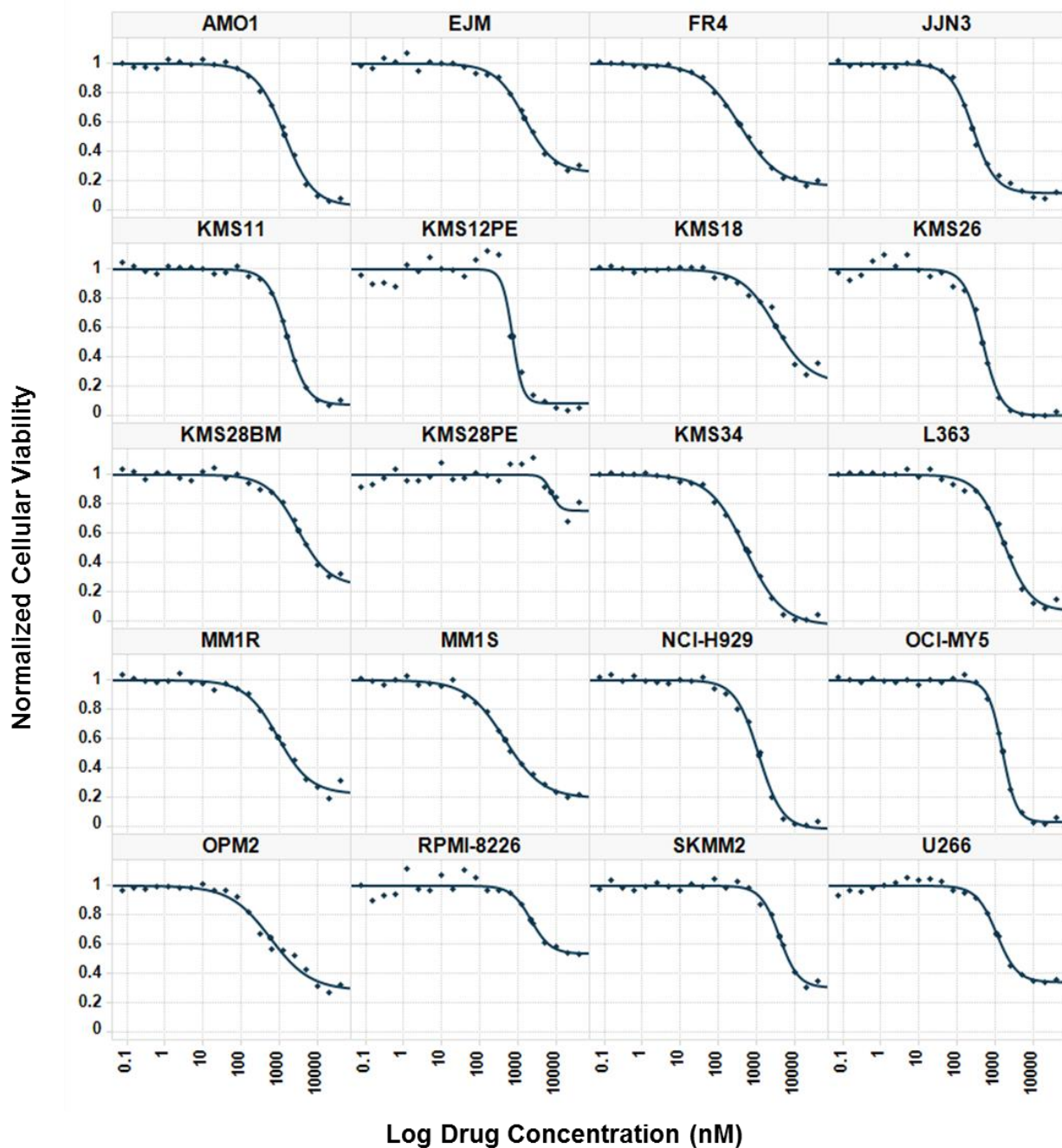


Figure S4. Normalized cellular viability of 20 human myeloma cell lines incubated for 72 hours with a 20-point 2-fold dilution starting at 40 μ M of YM201636.

Table S1. Sensitivity of 20 human myeloma cell lines (HMCL) to three PIKfyve inhibitors, APY0201, YM201636, and apilimod with mid-point EC₅₀ (nM) and maximum inhibition (MI) (%) after 72 hour incubation.

HMCL	APY0201		YM201636		Apilimod	
	EC ₅₀	MI	EC ₅₀	MI	EC ₅₀	MI
JJN3	15.14	100	256.75	98.9	558.99	100
KMS26	19.17	100	466.46	100	1072.22	100
KMS12PE	29.81	100	708.65	100	1531.67	100
H929	81.39	100	1123.22	100	8331.98	100
FR4	93.74	100	356.38	93.07	2970.29	100
KMS34	107.81	100	518.28	100	3010.38	100
MM1.R	113.61	83.04	903.89	87.5	> 10000	100
U266	114.27	100	1086.22	73.37	2309.89	71.86
SKMM2	132.66	100	3941.03	77.19	9120.94	100
AMO1	152.11	100	1357.13	100	2262.59	100
KMS11	527.02	100	1612.02	100	9024.59	100
MM1.S	572.74	100	442.24	89.2	> 10000	100
KMS28BM	600	100	3097.10	84.45	8949.04	100
L363	1387.84	100	1658.19	100	6885.42	100
MY5	2939.52	100	1529.19	100	> 10000	100
EJM	4000	100	1479.69	82.04	> 10000	100
KMS18	4776.99	100	3101.69	87.4	> 10000	100
KMS28PE	> 10000	100	7447.59	27.11	> 10000	87.36
OPM2	> 10000	100	575.64	79.54	10000	100
8226	> 10000	80	2160.33	51.8	> 10000	100

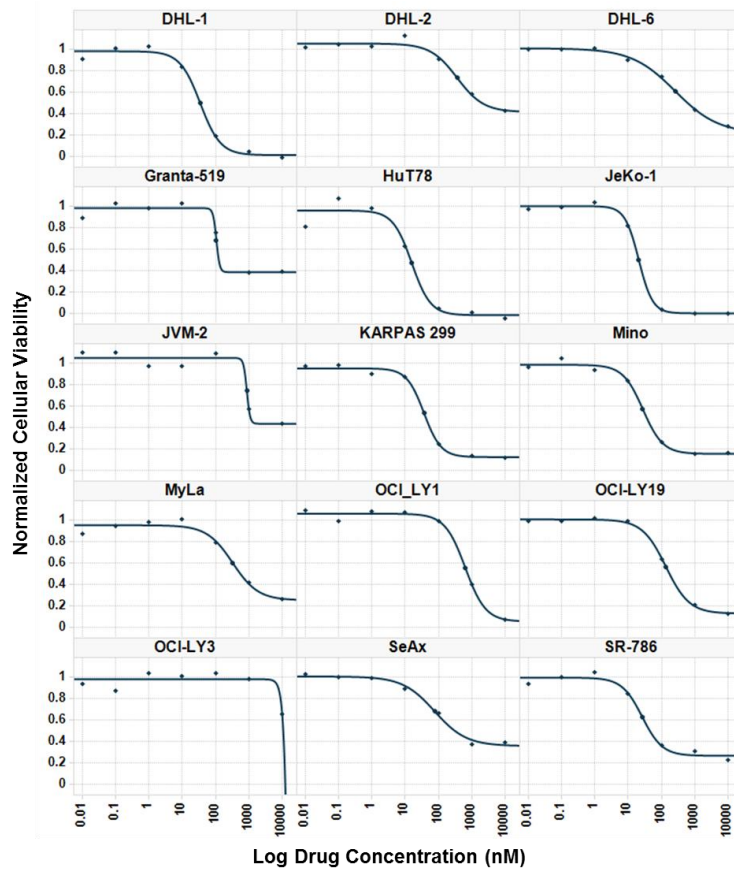


Figure S5. Normalized cellular viability of 15 non-Hodgkin's lymphoma (NHL) cell lines incubated for 72 hours with a 7-point 10-fold dilution starting at 10 μ M of APY0201.

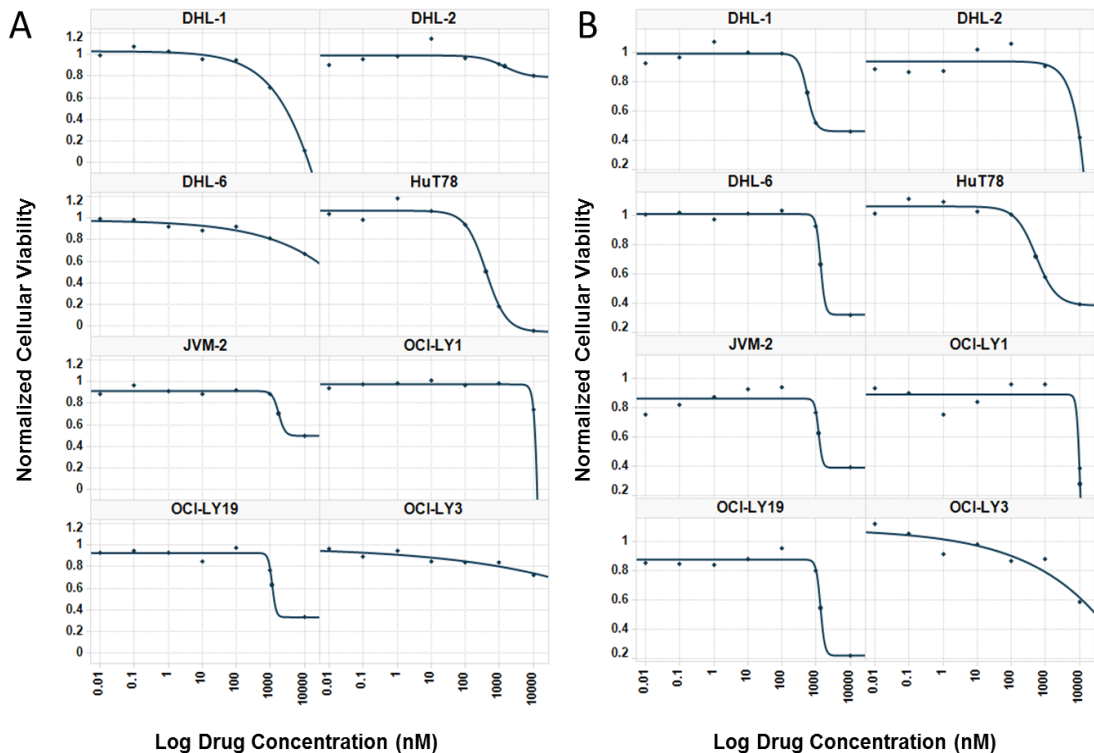


Figure S6. Normalized cellular viability of 8 non-Hodgkin's lymphoma (NHL) cell lines incubated for 72 hours with a 7-point 10-fold dilution starting at 10 μ M of apilimod (A) and YM201636 (B).

Table S2. Sensitivity of 15 non-Hodgkin's lymphoma (NHL) to three PIKfyve inhibitors, APY0201, YM201636, and apilimod with mid-point EC₅₀ (nM) and maximum inhibition (MI) (%) after 72 hour incubation.

Cell Line		APY0201		YM201636		Apilimod	
		EC ₅₀	MI	EC ₅₀	MI	EC ₅₀	MI
NHL	JVM2	1035.89	56.21	422.47	34.29	> 1000	20
	KARPAS299	36.11	87.91	NA	NA	NA	NA
	MYLA	323.13	74.95	NA	NA	NA	NA
	SR786	26.14	73.65	NA	NA	NA	NA
	MINO	27.49	84.88	NA	NA	NA	NA
	JEKO	20.61	100.00	NA	NA	NA	NA
	SEAX	75.87	64.46	NA	NA	NA	NA
	GRANTA	105.39	61.68	NA	NA	NA	NA
	HUT78	15.55	100.00	529.74	61.55	405.47	100.00
	DHL1	34.42	99.04	544.18	53.78	> 1000	90
	DHL2	108.55	62.93	Inactive	NA	> 1000	20
	DHL6	114.49	72.21	Inactive	NA	Inactive	NA
	LY1	162.61	97.93	Inactive	NA	Inactive	NA
	LY3	793.73	46.32	Inactive	NA	Inactive	NA
LY19	53.34	94.94	Inactive	NA	1452.26	59.25	

NA indicates not applicable

Table S3. Sensitivity of 15 *ex vivo* primary patient samples to two PIKfyve inhibitors, APY0201 and apilimod with mid-point EC₅₀ (nM) and maximum inhibition (MI) (%).

Primary Patient Samples	APY0201		Apilimod	
	EC ₅₀	MI	EC ₅₀	MI
Patient 112	400	50	40000	60
Patient 113	7.69	100	4000	100
Patient 114	179.36	71.8	40000	55
Patient 115	Inconclusive	NA	40000	100
Patient 116	50000	50	50000	50
Patient 118	28.11	60.3	40000	100
Patient 119	10.4	87.6	5500	100
Patient 120	5282.79	73.8	50000	100
Patient 121	1	100	1640.37	99.3
Patient 122	2126.32	68.6	13706.16	100
Patient 123	119.86	69.2	22617.56	100
Patient 124	4	90	500	100
Patient 125	658.33	20.13	18674.13	94.6
Patient 126	Inactive	NA	23313.89	74.8
Patient 128	262.04	41.6	17238.54	100

NA indicates not applicable

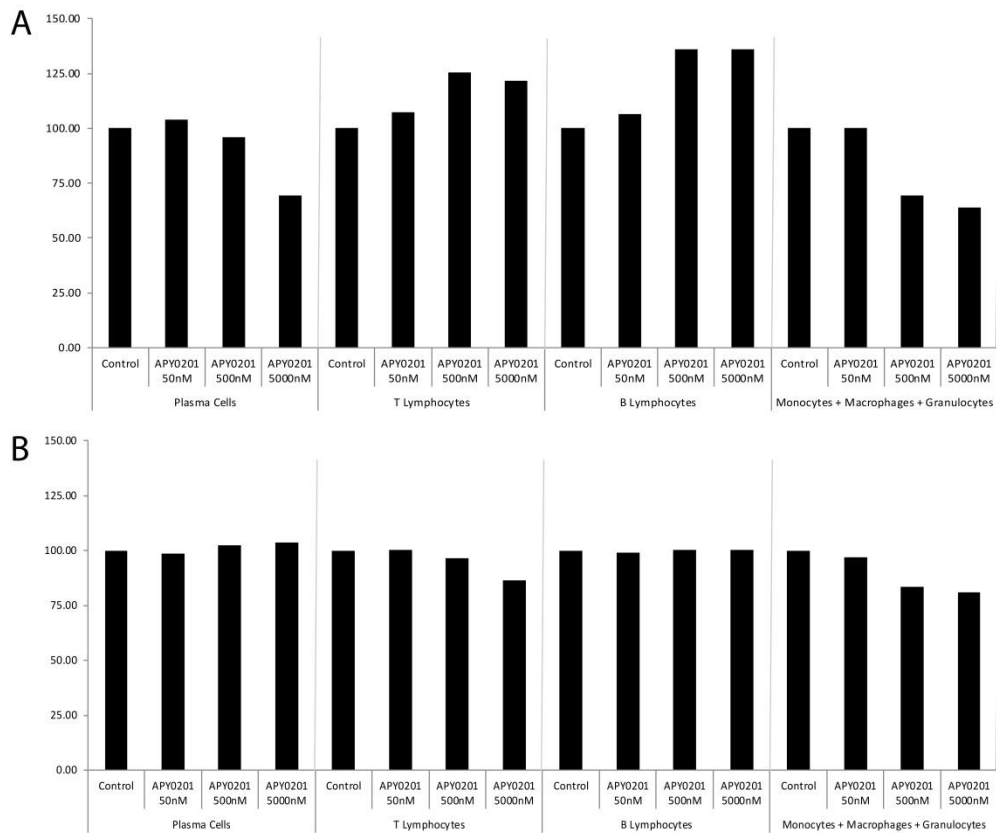


Figure S7. Viability (%) of leukocytes from two whole bone marrow primary patient samples incubated for 24 hours with increasing concentrations of APY0201 and stained with a viability dye, Annexin V, CD138, CD3, CD14, and CD19 in order to determine the cytotoxic profile of the drug in different cell populations.

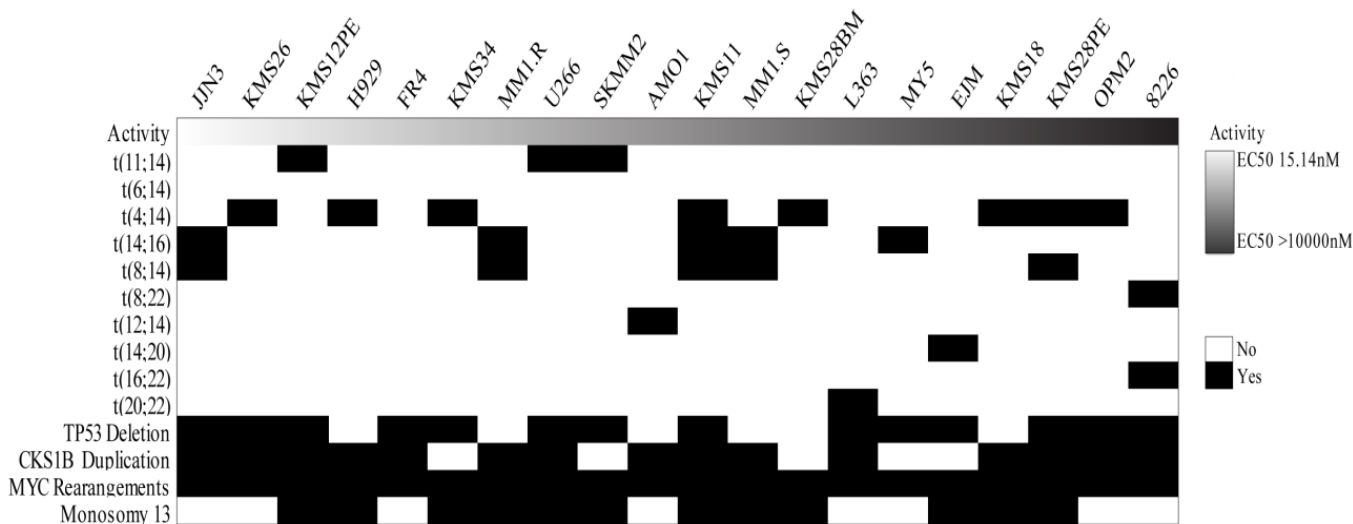


Figure S8. A global overview of data from the 20 HMCLs tested for APY0201 activity including t(11;14), t(6;14), t(4;14), t(14;16), t(8;14), t(8;22), t(12;14), t(14;20), t(16;22), t(20;22), TP53 deletion, CKS1B duplication, MYC rearrangements, and monosomy 13. IgH translocation and TP53 deletion information from the HMCLs was obtained through data publicly available at www.keatslab.org. CKS1B gain and monosomy 13 information was obtained through array-based comparative genomic hybridization (aCGH) data, and MYC rearrangement information was obtained through mate pair sequencing data.

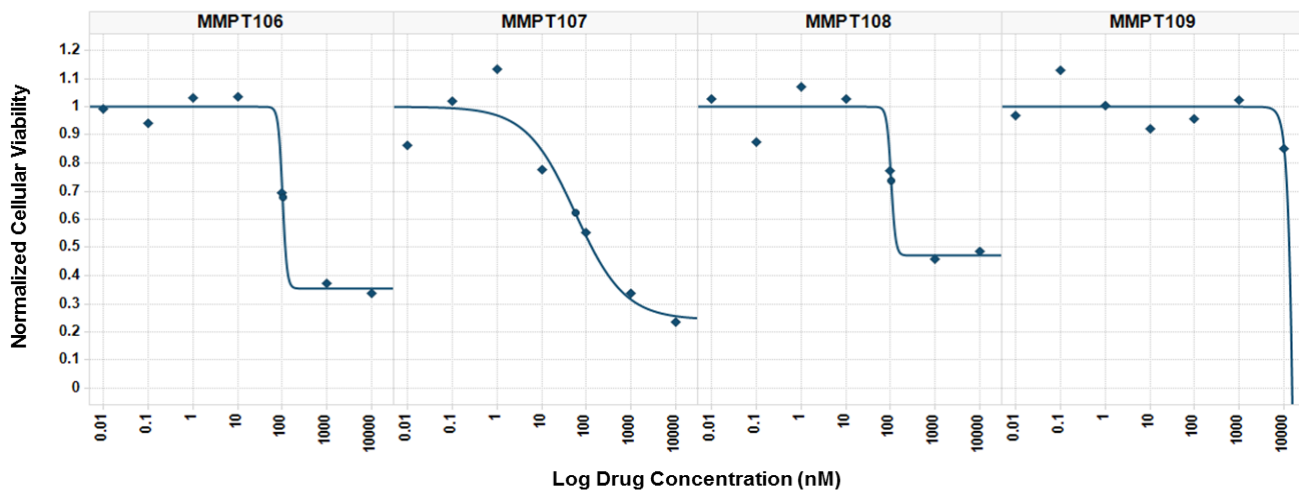


Figure S9. Normalized cellular viability of four primary patient samples incubated with a 7-point 10-fold dilution starting at 10 μ M of APY0201. Three samples were considered active (MMPT106, MMPT107, MMPT108) and MMPT109 was considered inactive.

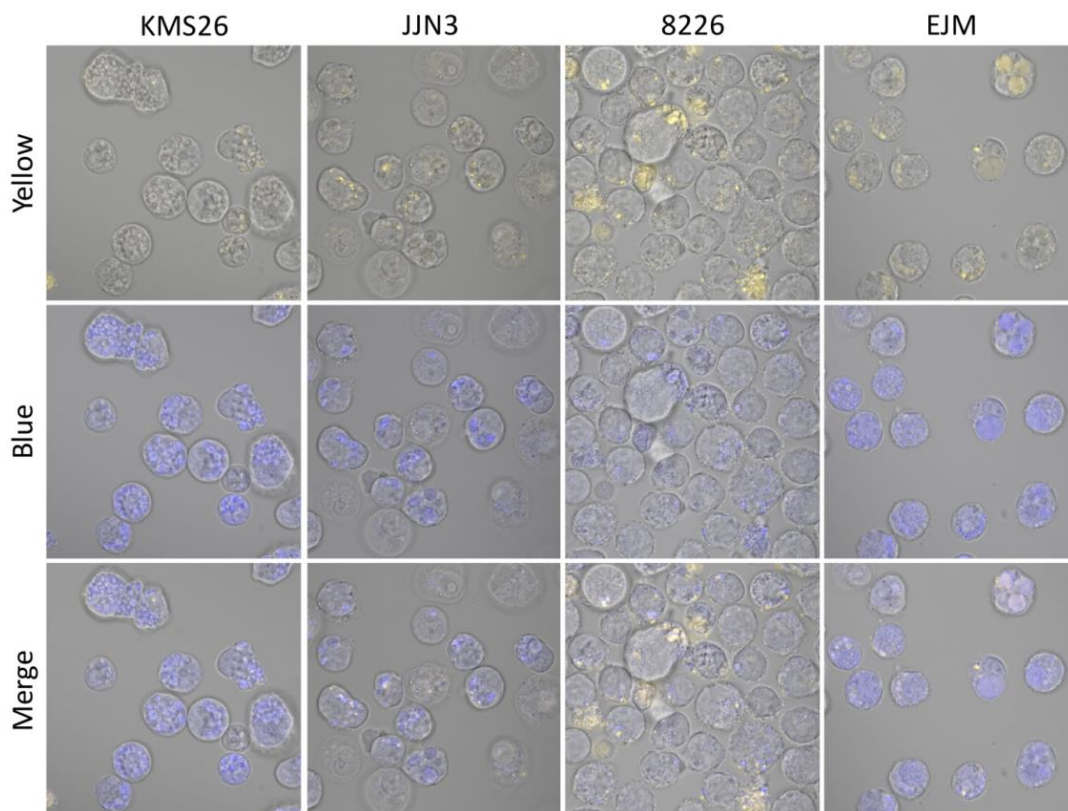


Figure S10. Two sensitive (KMS26 and JN3) and two resistant (RPMI-8226 and EJM) HMCLs treated with APY0201 at 100nM for 24 hours and 10 μ M of the LysoSensor Yellow/Blue DND-160 probe (Thermo Fisher Scientific #L7545). Images were obtained with a confocal microscope Zeiss LSM 800 (63X).