cancers

Supplementary Materials: Targeting the MYC Oncogene in Burkitt Lymphoma through HSP90 Inhibition

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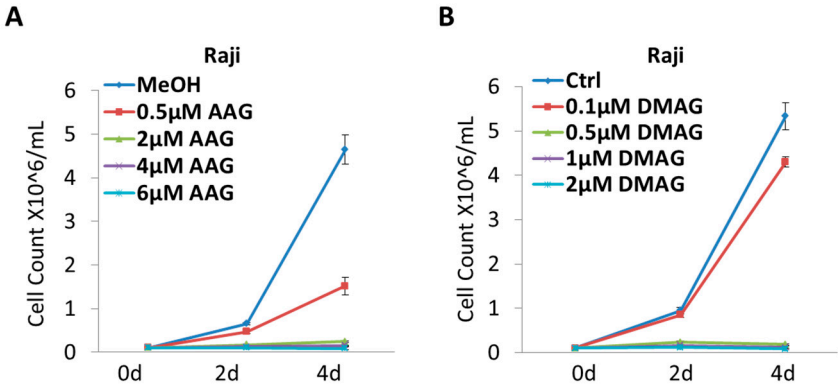

Figure S1. Dose response curve for 17-AAG and 17-DMAG treatment on Burkitt Lymphoma. Dose response growth curve on Raji Burkitt Lymphoma cell line with (**A**) 0.5-6μM 17-AAG compared to Methanol (MeOH) control; and (**B**) 0.1-2μM 17-DMAG compared to untreated control.

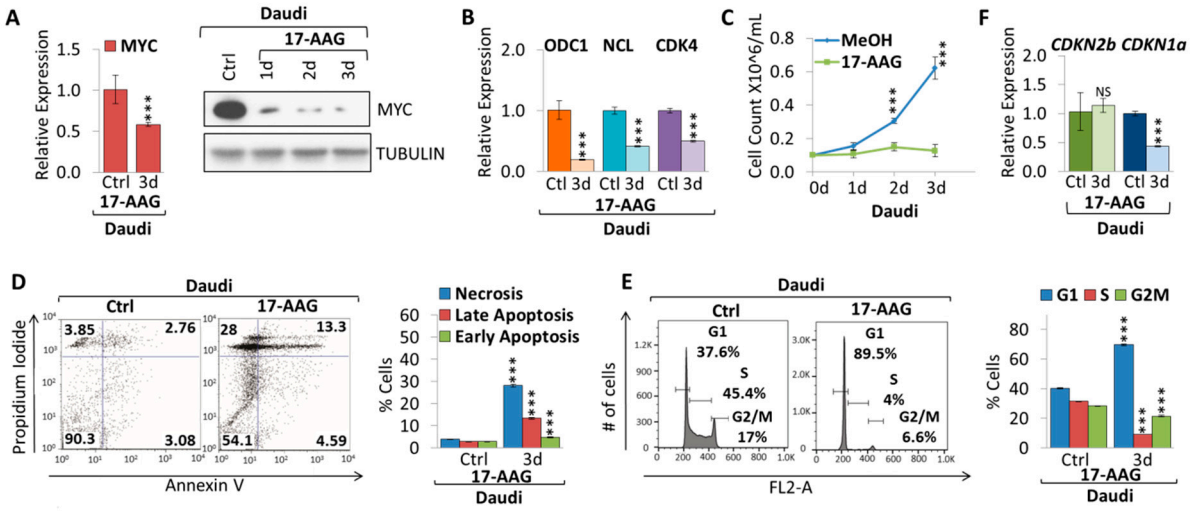

Figure S2. 17-AAG treatment downregulates MYC expression and caused decreased tumor cell proliferation, apoptosis, and cell cycle arrest in Daudi cells. (**A**) RT-qPCR and WB of MYC expression upon 4 μM 17-AAG treatment over the course of four days; Tubulin is used as a WB loading control. (**B**) RT-qPCR of canonical MYC target genes: *CDK4*, *NCL*, and *ODC1* upon three days treatment of 4 μM 17-AAG or methanol control. (**C**) Growth curve of cells treated with methanol control or 4 μM 17-AAG over the course of four days. (**D**) Flow cytometry profile of AnnexinV staining (X axis) and PI (Y axis) upon three days treatment with 4 μM 17-AAG. The lower right quadrant indicates the percentage of early apoptotic cells in each condition; the upper right quadrant indicates the percentage of late apoptotic cells; the upper left quadrant indicates percentage of necrotic cells; and the left lower quadrant indicates percentage of live/non-apoptotic cells. Apoptotic cells (Annexin V-positive cells) are displayed as the percentage of gated cells. (**E**) Flow cytometric cell cycle analysis

using propidium iodide (PI) staining upon three days treatment with 4μM 17-AAG. Cell cycle distribution (G1, S and G2/M) are displayed in percent. (**F**) RT-qPCR of *CDKN2b* and *CDKN1a* upon three day treatment of 4 μM 17-AAG or methanol control. RT-qPCR was normalized to *RPL13a;* Tubulin was used as a loading control for WB. Error bars represent mean ± SEM; n = 3; two-tailed Student's *t*-test: NS = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

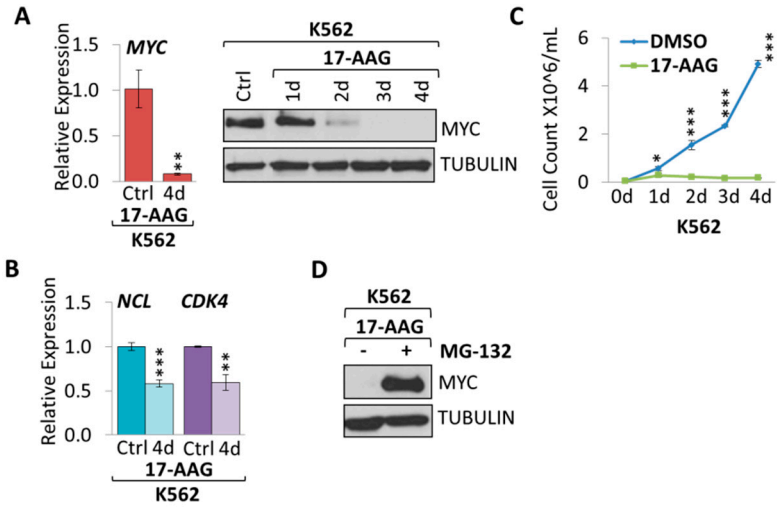

Figure S3. 17-AAG treatment causes MYC downregulation and proteasomal degradation of MYC protein in Chronic Myeloid Leukemia. (**A**) RT-qPCR and WB of MYC expression upon 4 μM 17-AAG treatment or DMSO control over the course of four days. (**B**) RT-qPCR of canonical MYC target genes: *CDK4* and *NCL*, upon four days treatment of 4 μM 17-AAG or DMSO control. (**C**) Growth curve of cells treated with DMSO control or 4 μM 17-AAG over the course of four days. (**D**) WB of MYC expression upon 4 μM 17-AAG and 10 μM MG-132 combination treatment over four hours. RT-qPCR was normalized to *RPL13a;* Tubulin was used as a loading control for WB. Error bars represent mean ± SEM; n = 3; two-tailed Student's *t*-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S1. RT-qPCR primers

Gene	Forward	Reverse
Hs *MYC*	CTGCGACGAGGAGGAGAA	GGCAGCAGCTCGAATTTCTT
Hs *CDK4*	ATGGCTACCTCTCGATATGAGC	CATTGGGGACTCTCACACTCT
Hs *NCL*	GCACCTGGAAAACGAAAGAAGG	GAAAGCCGTAGTCGGTTCTGT
Hs *ODC1*	TTTACTGCCAAGGACATTCTGG	GGAGAGCTTTTAACCACCTCAG
Hs *CDKN1A*	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC
Hs *CDKN2B*	GATGTGCAAGCGACGACAGA	GAGCAAAGGCCAGCATCCT
Hs *RPL13A*	CTGGAAGATGGTCGTACCCTG	GGTCTTGCCAGTGAGTGTCT

Table S2. ChIP-qPCR tiling primers

Amplicon	Relative/Absolute Location	Forward	Reverse
1	Chr14. 105,862,121/ 105,862,226	AGGTCACCGCGAGAGTCTAT	GCACTTTCAGATCTGGGCCT
2	Chr14. 105,582,220/ 105,582,343 and 105,701,268/ 105,701,391	GCCAGGTCTCGACTTAGCAC	TGGGGTACAAGAGGCTTCAG
3	Chr14. 105,575,299/ 105,575,593 and 105,696,320/ 105,696,508	TCCAGAAATAGCTTGCACGA	AGTAACCCAAGTGGGCCTGT
4	Chr14. 105,566,448/ 105,566,625 and 105,686,292/ 105,686,469	GGCTGGACACACTAGCAGGT	CTGCTCTGTGGTTCCCATTT
5	-2,163bp/ -2,255bp from *MYC* TSS	AACACTTGAACGCTGAGCTG	CCACCACCTCCAAAAGAGAA
6	-688bp/ -785bp from *MYC* TSS	GAGCAGCAGAGAAAGGGAGA	CAGCCGAGCACTCTAGCTCT
7	-438bp/ -534bp from *MYC* TSS	TTTATAATGCGAGGGTCTGGA	AGAAGCCCTGCCCTTCTC
8	-240bp/ -387bp from *MYC* TSS	GGGATCGCGCTGAGTATAAA	CCTATTCGCTCCGGATCTC
9	754bp/ 920bp from MYC TSS	GGGATCGCGCTGAGTATAAA	CCTATTCGCTCCGGATCTC
10	1,820bp/ 1,917bp from *MYC* TSS	AGGTGTTAGGACGTGGTGTTG	CCCTGGTTTTTCCAAGTCAA
11	2,881bp/ 2,996bp from *MYC* TSS	CGGTGCAGCCGTATTTCTAC	CAGCAGCTCGAATTTCTTCC
12	3,576bp/ 3,670bp from *MYC* TSS	AGCGACTCTGGTAAGCGAAG	AGTGGCCCGTTAAATAAGCTG

13	4,990bp/ 5,083bp from *MYC* TSS	CGATGTTGTTTCTGTGGAAAAG	GCTGTGAGGAGGTTTGCTGT
14	5,425bp/ 5,522bp from *MYC* TSS	AGCAGAGGAGCAAAAGCTCA	ACGCACAAGAGTTCCGTAGC