Unlocking the therapeutic potential of selective CDK7 and BRD4 inhibition against multiple myeloma cell growth

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Supplemental Methods

Western Blot. Treated MM cells were lysed using RIPA lysis buffer (Boston Bio Products, #BP-115) supplemented with a protease inhibitor cocktail (Thermo Fisher Scientific, #78440). Protein concentrations were measured by the BCA Protein Assay Kit (Thermo Fisher Scientific, #23227) and separated by SDS-PAGE. The following primary antibodies were employed in this study: Rb (sc-73598), phospho-Rb (s780) (CST, #8180), phospho-Rb (s795) (CST, #9301), phospho-Rb (s807/811), p-CDK4 (ABclonal, AP-0593), CDK4(CST, #12790), Cleaved-caspase3 (CST, #9661), MYC (sc-40), and Tubulin (CST, #2128).

Supplemental Figure Legends.

Figure S1. Dual inhibition synergistically inhibits MM cell growth. A. A panel of 10 MM cell lines was treated with YKL-5-124 or JQ1 alone or in combination, and cell survival was assessed by CTG uptake assay. Data are shown as % of cell viability compared to untreated cells. Data represent mean±SD; n=3. **B.** PBMCs from healthy donors were treated with different concentrations of YKL-5-124 or JQ1 alone or in combination for 72 hours, and cell viability was assessed by CTG uptake. Data are presented as % of cell viability compared to untreated cells. Data represent mean±SD; n=3. **C.** XG1 cells were cultured in the presence of different concentrations of YKL-5-124 with or without 50nM JQ1 for 72h, and apoptotic cells were assessed by flow cytometric analysis following Annexin V+/PI staining. **D.** H929 and JJN3 cells were treated with 100nM YKL-5-124 or 100nM JQ1 alone or in combination for 24 hours, and WB analysis was performed using indicated antibodies against cleaved caspase3, with Tubulin as a loading control. **E-F.** A panel of MM cells was treated with a low dose of Palbociclib or JQ1 alone or in combination, and cell viability was assessed by CTG. Data from a representative panel of cell lines are shown as % of cell viability compared to untreated cells (**E**). Data represent mean ± SD; n=3. Synergism analysis was performed with the Calcusyn software (**F**). The gray area delineates potent synergistic combinations. CI = 1 means additive effect, CI < 1 means synergistic effect, and CI > 1 means antagonistic effect.

Figure S2. Targeting CDK7, alone and in combination, halts WM cell growth. A. Three WM cell lines were treated with different doses of YKL-5-124 for 24-72 hours, and cell growth was assessed by CTG assay. Data represent mean ± SD; n=3. Data from these WM cells are shown as % of cell viability compared to untreated cells. **B.** Primary WM cells from patients were treated with low doses of YKL-5-24 for 24 hours, and cell

growth was assessed by CTG assay. Data from these WM cells were shown as % of cell viability compared to untreated cells. Data represent mean ± SD; n=3. ** p <0.01 and **** p <0.0001. **C.** RPCIWM1 cells were treated with DMSO or YKL-5-124 for 24h, and then subjected to RNA-seq analysis. Gene Sets Enrichment Analysis of differentially expressed genes using Hallmark gene set in panel. **D-E.** BCWM1 and MWCL1 were treated with low doses of YKL-5-124 and JQ1 for 72 hours, and cell growth was assessed by CTG uptake assay (**D**). Data was shown as % of cell viability compared to untreated cells. Data represent mean±SD; n=3. Synergism analysis was performed with the Calcusyn software (**E**). Data represent the average over 3 replicates. CI = 1 means additive effect, CI < 1 means synergistic effect, and CI > 1 means antagonistic effect.

Figure S3. MYBL2 is oncogenic in MM. A. CRISPR screen score (CSS) for MYBL2 in CRISPR-Cas9 KO screening in 16 MM cell lines. **B-C.** Correlation of MYBL2 with overall survival (OS) or event-free survival (EFS) in the MMRF CoMMpass (**B**) and GSE2685 patient datasets (**C**).

Figure S4. Therapeutic potential of combination therapy ex vivo and in vivo. A. Primary MM cells from patients were treated with a low dose of Palbociclib and JQ1 for 24 hours, and cell viability was assessed by CTG uptake assay. Data represent mean ±SD; n=3. *** p < 0.001 and **** p < 0.0001. **B.** PBMCs and primary MM cells were treated with a low dose of Palbociclib and JQ1 for 24 hours, and cell viability was assessed by CTG assay. Data represent mean±SD; n=3. **C.** Sub-lethally irradiated SCID mice were injected subcutaneously with H929 cells. Mice were randomized to receive YKL-5-124 (2.5 mg/kg, i.p, 5 days/week), JQ1 (50mg/kg, 2 days/week) or a combination for 2 weeks. Tumor volume was evaluated by caliper measurement. Fold change increase of tumor volume compared to start of treatment (Day 1) (mean +/- SD) are shown. p-values indicate significant differences between groups. * p <0.05. **D.** Sub-lethally irradiated SCID mice were injected subcutaneously with H929 cells. Mice were randomized to receive Palbociclib (25 mg/kg, i.p, 3 days/week), JQ1 (50mg/kg, 2 days/week) or a combination for 2 weeks. Tumor volume was evaluated by caliper measurement. p-values indicate significant differences between groups. * p<0.05.

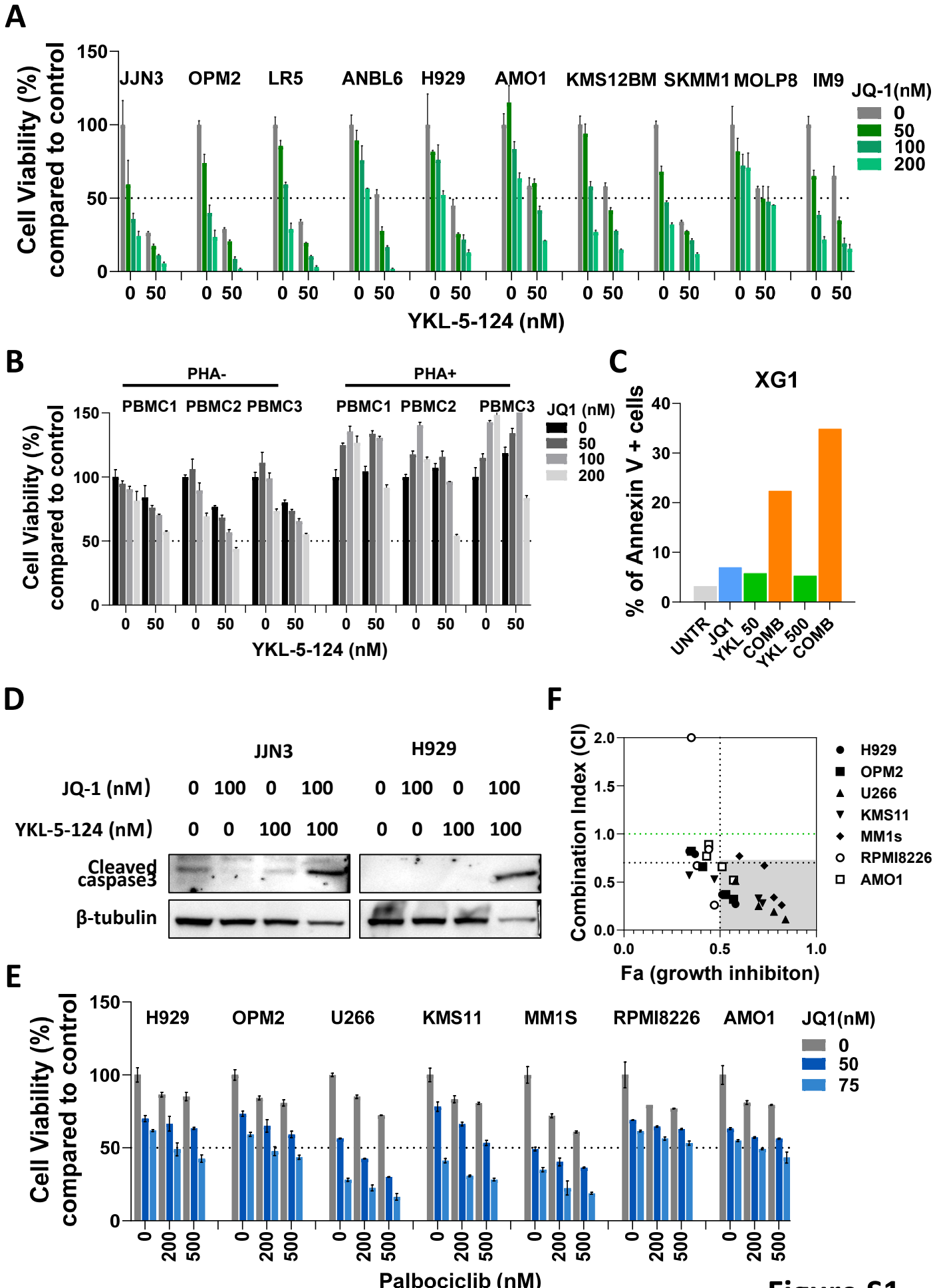
A
Cell Viability (%) compared to control
JJN3
OPM2
LR5
ANBL6
H929
AMO1
KMS12BM
SKMM1
MOLP8
IM9
JQ-1(nM)
0
50
100
200
YKL-5-124 (nM)
B
PHA-
PHA+
PBMC1
PBMC2
PBMC3
JQ1 (nM)
C
XG1
% of Annexin V + cells
UNTR
JQ1
YKL 50
COMB
YKL 500
COMB
D
JJN3
H929
JQ-1 (nM)
YKL-5-124 (nM)
Cleaved caspase3
β-tubulin
F
Combination Index (CI)
Fa (growth inhibiton)
H929
OPM2
U266
KMS11
MM1s
RPMI8226
AMO1
E
H929
OPM2
U266
KMS11
MM1S
RPMI8226
AMO1
JQ1(nM)
Palbociclib (nM)

Figure S1

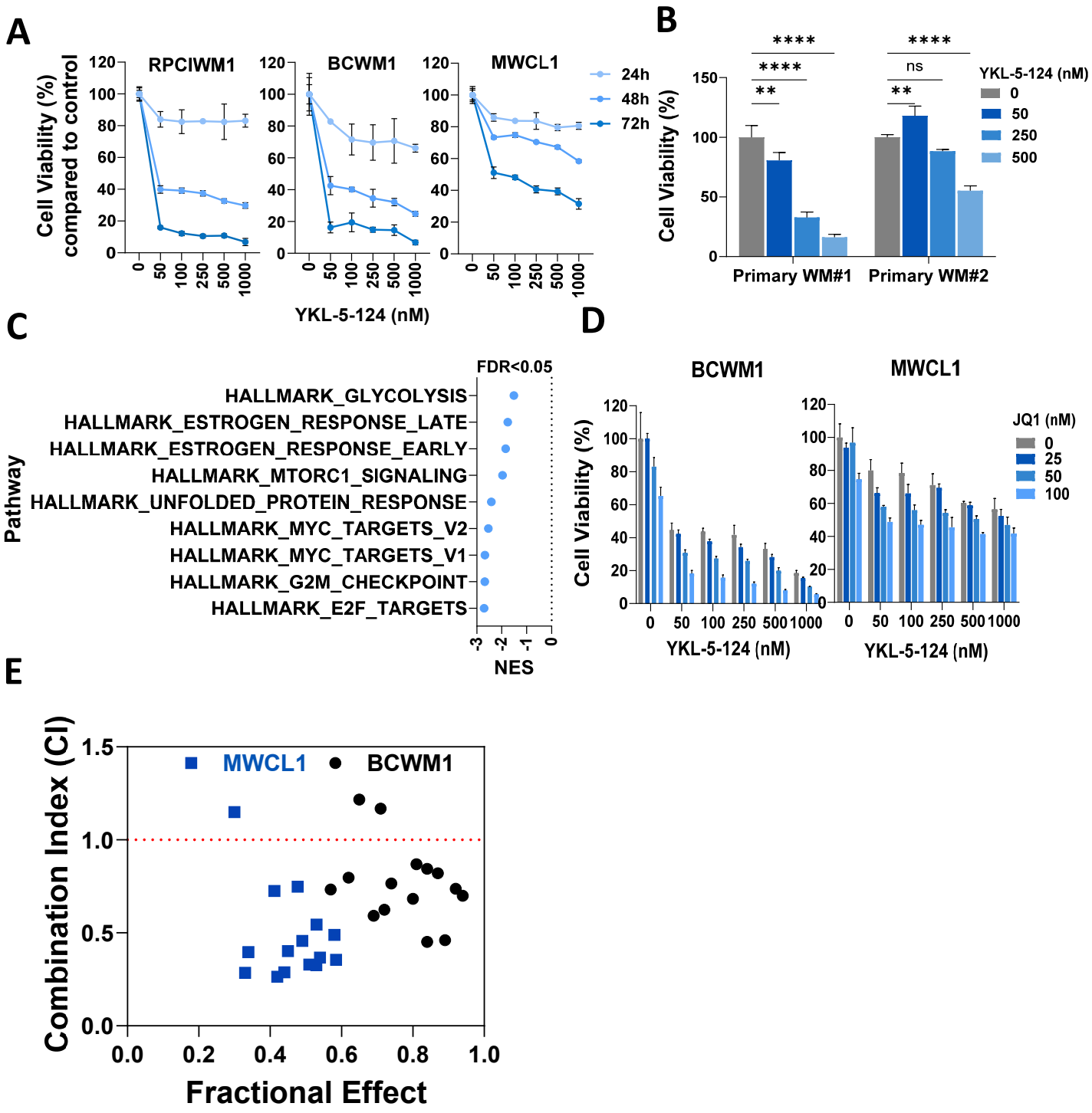
A
RPCIWM1
BCWM1
MWCL1
Cell Viability (%) compared to control
YKL-5-124 (nM)
24h
48h
72h
B
Cell Viability (%)
YKL-5-124 (nM)
0
50
250
500
Primary WM#1
Primary WM#2
****
**
ns
C
Pathway
FDR<0.05
HALLMARK_GLYCOLYSIS
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_MTORC1_SIGNALING
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_MYC_TARGETS_V2
HALLMARK_MYC_TARGETS_V1
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
NES
D
BCWM1
MWCL1
Cell Viability (%)
YKL-5-124 (nM)
JQ1 (nM)
0
25
50
100
E
Combination Index (CI)
MWCL1
BCWM1
Fractional Effect

Figure S2

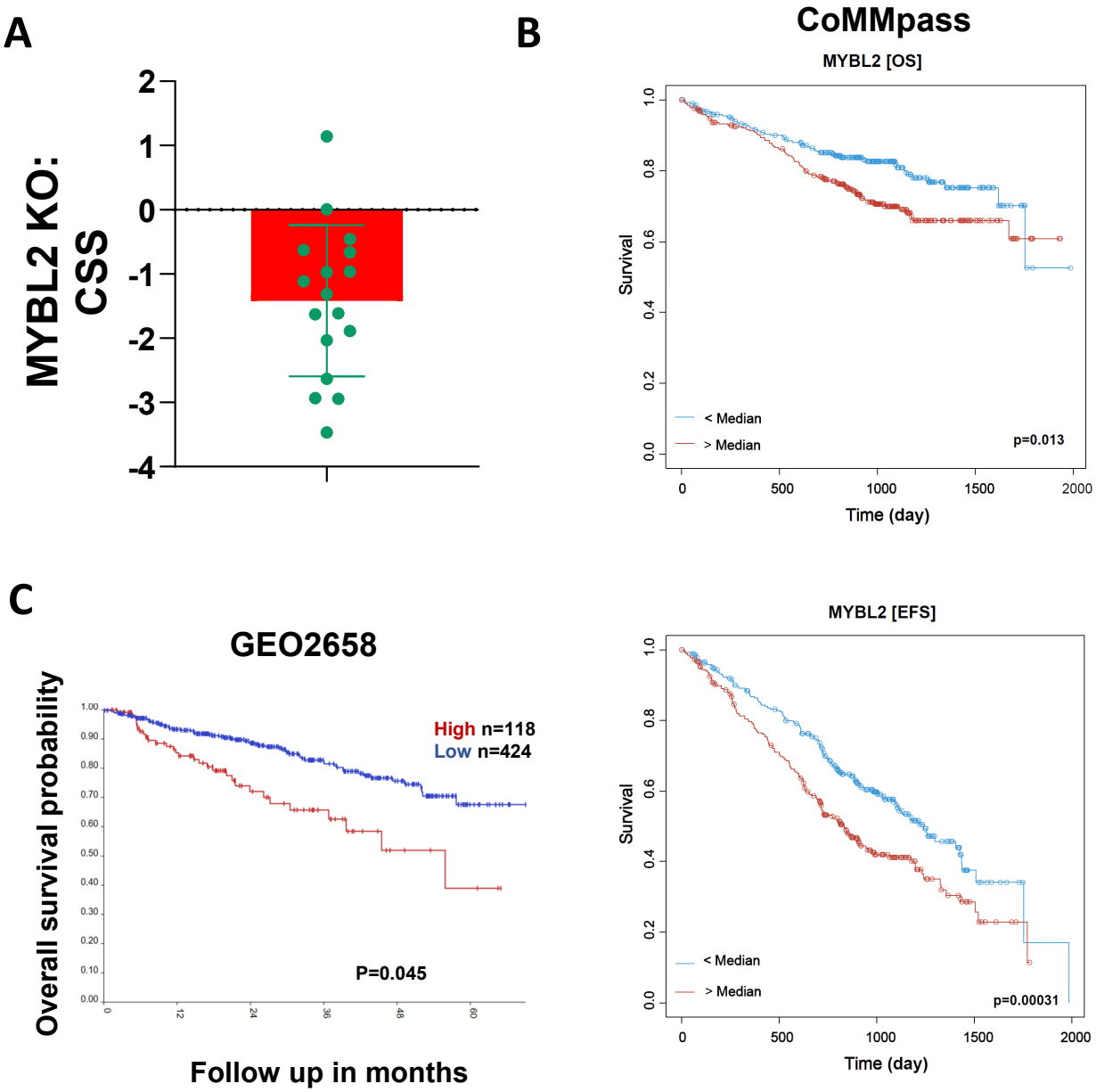
A
MYBL2 KO:
CSS
2
1
0
-1
-2
-3
-4
B
CoMMpass
MYBL2 [OS]
Survival
1.0
0.8
0.6
0.4
0.2
0.0
< Median
> Median
p=0.013
0
500
1000
1500
2000
Time (day)
C
GEO2658
Overall survival probability
High n=118
Low n=424
P=0.045
0
12
24
36
48
60
Follow up in months
MYBL2 [EFS]
Survival
< Median
> Median
p=0.00031
Time (day)

Figure S3

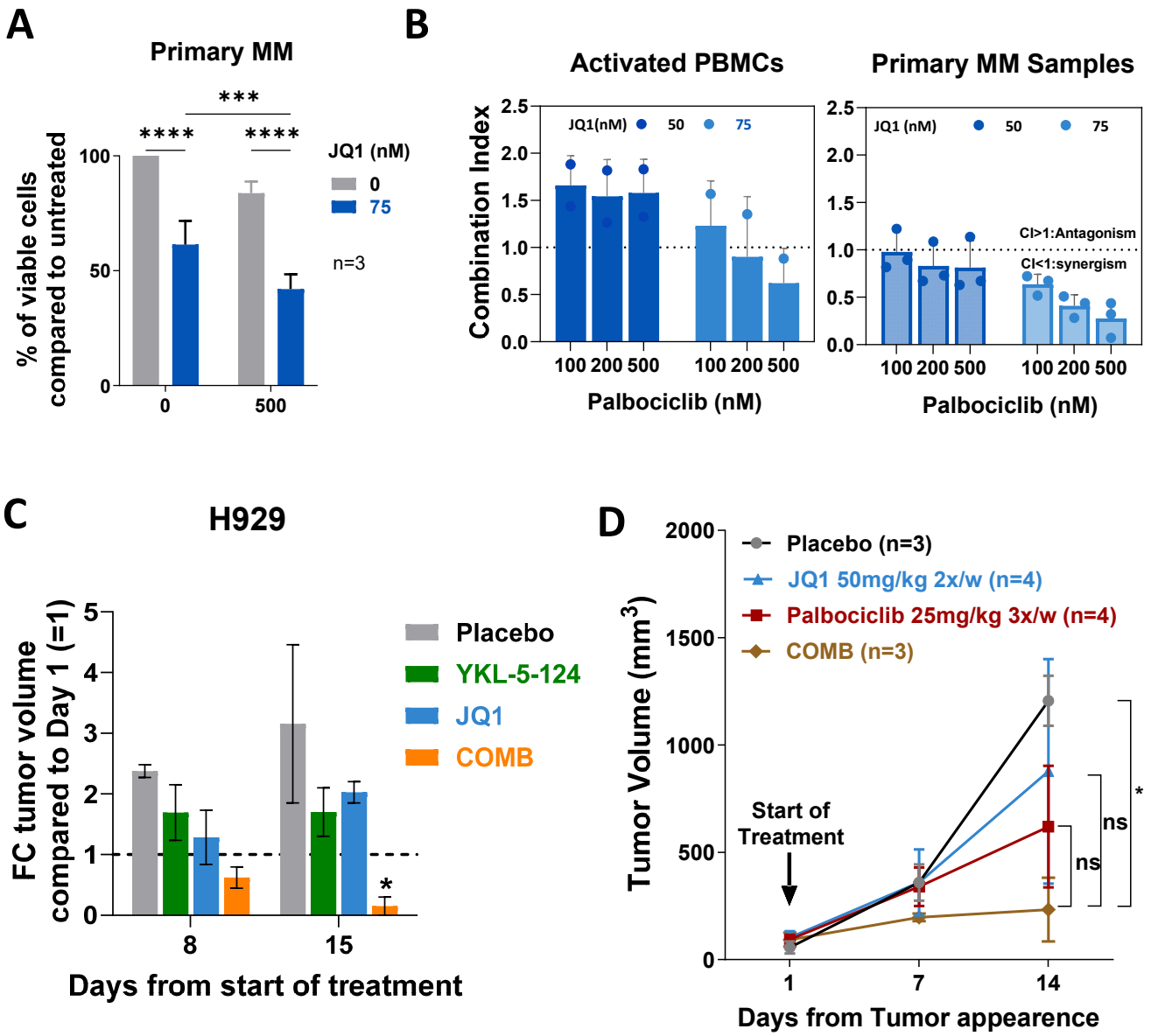
A
Primary MM
***
****
****
% of viable cells compared to untreated
100
50
0
0
500
JQ1 (nM)
0
75
n=3
B
Activated PBMCs
Primary MM Samples
Combination Index
JQ1(nM) 50 75
JQ1 (nM) 50 75
CI>1:Antagonism
CI<1:synergism
100 200 500
100 200 500
Palbociclib (nM)
Palbociclib (nM)
C
H929
FC tumor volume compared to Day 1 (=1)
Placebo
YKL-5-124
JQ1
COMB
*
8
15
Days from start of treatment
D
Tumor Volume (mm3)
Placebo (n=3)
JQ1 50mg/kg 2x/w (n=4)
Palbociclib 25mg/kg 3x/w (n=4)
COMB (n=3)
Start of Treatment
ns
ns
*
1
7
14
Days from Tumor appearence

Figure S4