Supplementary Information.

Supplementary Figure 1. YWHAE/14-3-3ε expression levels correlates with PI sensitivity in MM cell lines. A) Pearson correlation analysis of 14-3-3ε protein expression level and IC50 for BTZ treatment in a panel of MM cell lines. B) Pearson correlation analysis of 14-3-3ε protein expression level and IC50 for CFZ treatment in a panel of MM cell lines. C) 14-3-3ε protein expression level in ANBL-6 WT and ANBL-6/V10R cell lines was investigated by western blot analysis. Tubulin was used as loading control. 14-3-3ε /Tubulin ratio is shown in the top panel. D) 14-3-3ε protein expression level in a panel of MM cell lines was investigated by western blot analysis. E) 14-3-3ε protein expression level was investigated in CD19+ cells from PBMC of healthy donors (n=4) and CD138+ MM cells from BM aspirates of MM patients (n=2). GAPDH was used as loading control.

Supplementary Figure 2. 14-3-3ε modulation impacts sensitivity to PIs in MM cells. A-B) H929, or JJN3 and AMO1 MM cells were infected with either scrambled (pLKO.1) or 14-3-3ε -targeted shRNA and selected with puromycin for 72 hours. Expression of 14-3-3ε protein was evaluated by western blot (A). Cell viability was assessed by CTG and expressed as fold change from untreated cells (B). Data represent mean ±SD from three experiment performed in triplicates. C). JJN3 cells were infected with either scrambled (pLKO.1) or 14-3-3ε -targeted shRNA and selected with puromycin for 72 hours. Transduced cells were treated with 5nM of BTZ for 24 hours. Protein was extracted and expression of Noxa protein was evaluated by western blot. D-E). H929 were infected with either scrambled (pLKO.1) or 14-3-3ε -targeted shRNA and selected with puromycin for 72 hours. Transduced cells were treated with different concentrations of BTZ for 105 mins, proteasome activity (Chymotrypsin-Like, Trypsin-Like and Caspase-Like) were measured with Proteasome-Glo™ Cell-Based Assays kit according to the manufacturer's protocol. Data represent mean ±SD from three experiment performed in triplicates. Data were analyzed using unpaired Student t tests: *p$\leqslant$0.05, **P$\leqslant$0.01, ***P$\leqslant$0.001, ****P$\leqslant$0.0001.

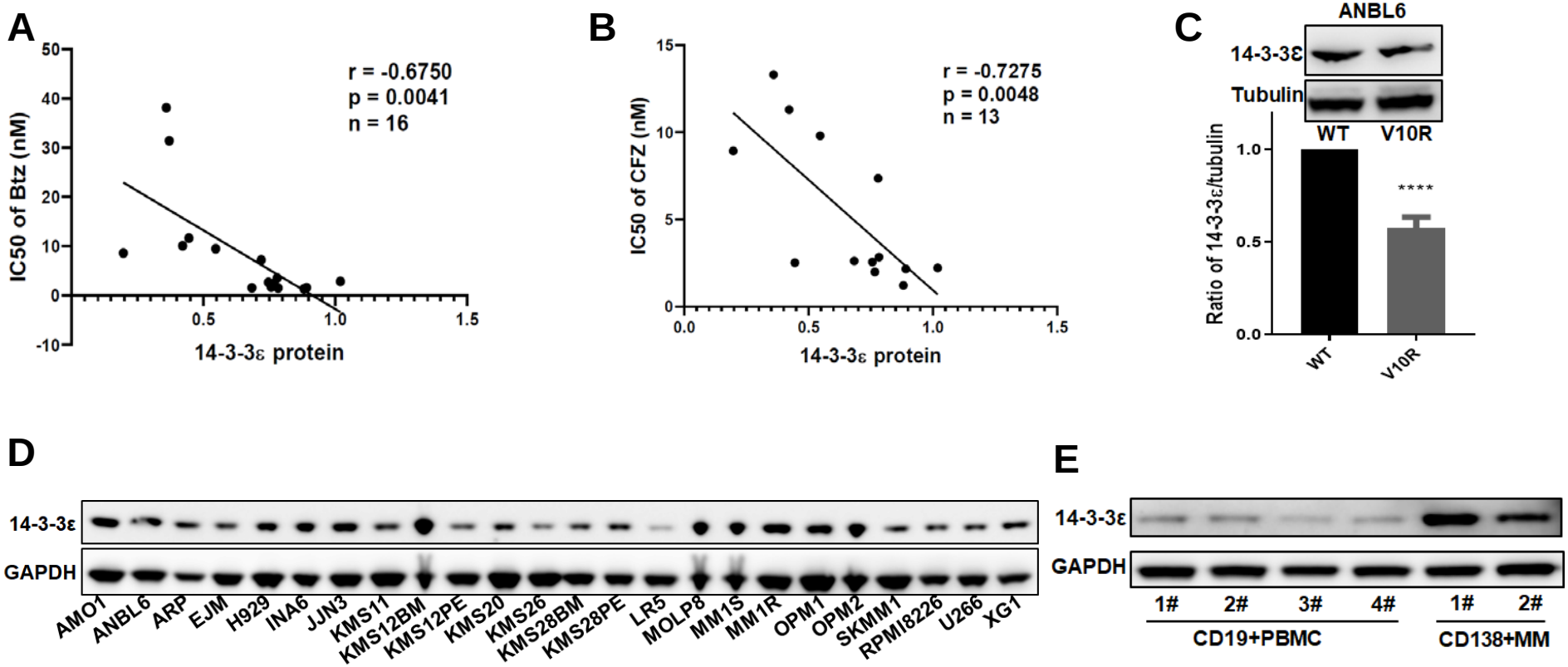
Supplementary Figure 3. 14-3-3ε overexpress or addback in MM cells. A) ANBL-6 WT, ANBL-6 V10R, KMS20, KMS26, LR5, and SKMM1 MM cells were infected with either pLenti6-empty (empty) or pLenti6-FLAG-YWHAE overexpression (OE) plasmid (14-3-3ε). Expression of

14-3-3ε protein was evaluated by western blot. B) KMS20, KMS26, LR5, SKMM1 MM cells and KMS11 with 14-3-3ε KO cells were infected with either pLenti6-empty (empty) or pLenti6-FLAG-YWHAE OE plasmid (14-3-3ε). Cell viability was assessed by CTG and expressed as fold change from untreated cells. Data represent means±SD from three experiment performed in triplicates. Data were analyzed using unpaired Student t tests: *p≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001.

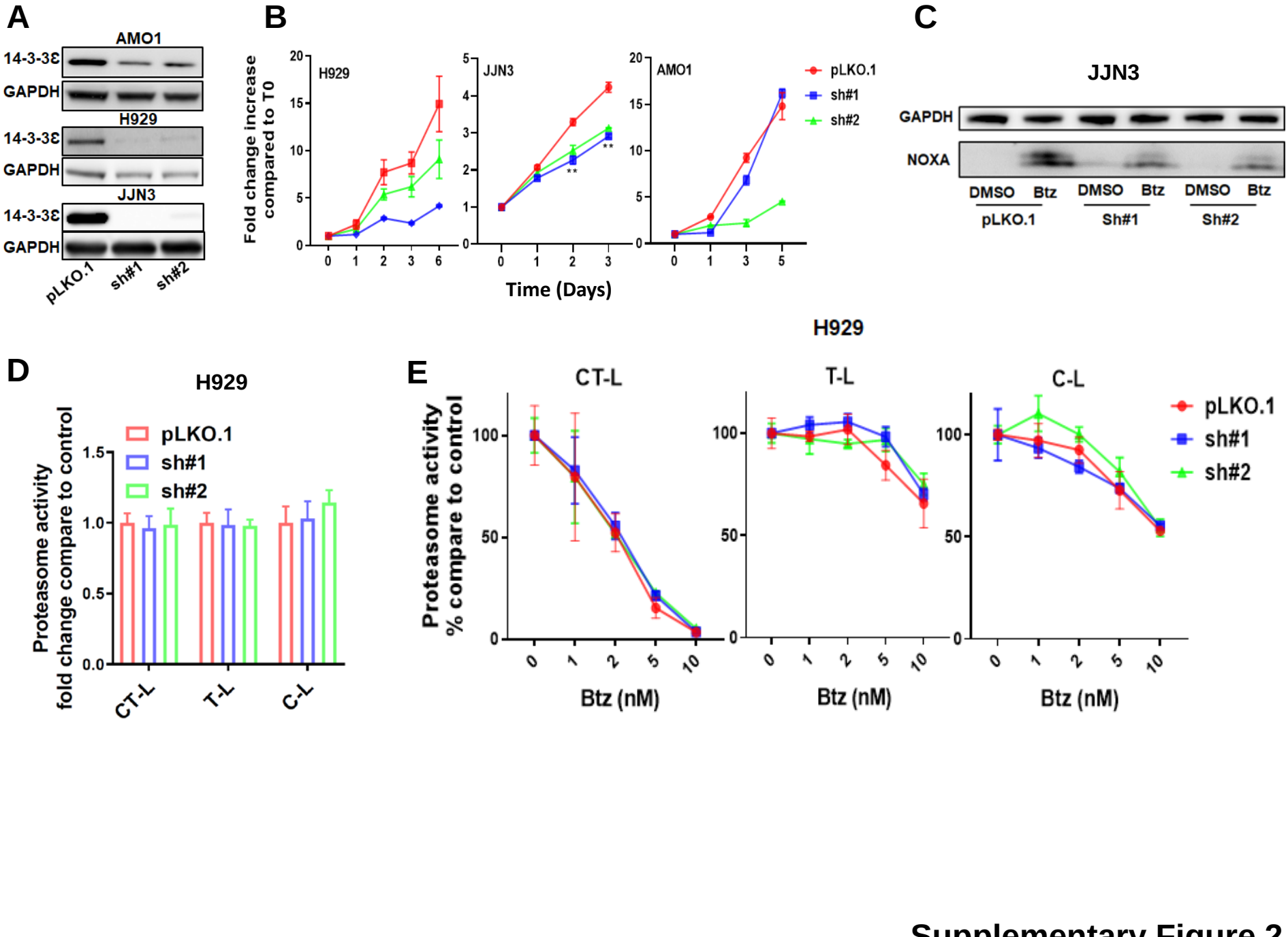
Supplementary Figure 4. 14-3-3ε interacts to and impacts mTORC1 signaling pathway. A) Anti-FLAG antibody was used to pull down 14-3-3ε binding proteins in H929 KO cells expressing pLenti6-FLAG-YWHAE plasmid. Cell lysate from H929 with YWHAE WT cells was used as negative control. Pull-down products were subjected to mass-spectrometry. Detailed 14-3-3ε interacting proteins involved in PI3K-AKT-MTOR, MTORC1 and UPR signaling pathways analyzed by hallmark GSEA. B). JJN3 cells were infected with either scrambled (pLKO.1) or 14-3-3ε -targeted shRNAs and selected with puromycin for 72 hours. Alive cells were collected, RNA was extracted and then HTA2.0 was performed. Hallmark gene set enrichment analysis (GSEA) of common downregulated genes in YWHAE KD cells compared to control cells.

Supplementary Figure 5. Depletion of YWHAE inhibits translation initiation complex formation. A). Western blots analysis in KMS12BM cells infected with either scrambled (pLKO.1) or 14-3-3ε-targeted shRNA. B) Western blot analysis in H929 cells infected with either scrambled (pLKO.1) or 14-3-3ε-targeted shRNA was performed using indicated mAbs. GAPDH or Tubulin were used as loading control. GAPDH or Tubulin ratio is shown in the right panel. C). Western blots analysis in H929 MM cells infected with either scrambled (pLKO.1) or two 14-3-3ε-targeted shRNAs (left panel); and in H929 KO cells expressing pLenti6-empty (empty) or pLenti6-FLAG-YWHAE OE plasmid (14-3-3ε) (right panel). One representative blot of two is shown. D) Protein synthesis was analyzed by flow cytometry in KMS20 MM cells infected with either pLenti6-empty (empty, red) or pLenti6-FLAG-YWHAE OE plasmid (14-3-3ε, blue) and treated with cycloheximide (CHX), and expressed as percentage compared to control cells. E) Flow cytometry analysis of clonal cytoplasmic kappa light chain expression in KMS11 KO cells expressing pLenti6-empty (empty) or pLenti6-FLAG-YWHAE OE plasmid (addback).

Supplementary Figure 6. 14-3-3ε expression is significantly lower in MM (patients and cell lines) with del17p and correlates with survival. A) YWHAE mRNA expression in CD138+ MM cells from bone marrow aspirates of 11 MM patients was evaluated by q-PCR. We established two groups with significantly different 14-3-3ε expression (superior and inferior to the median respectively). B-C) Expression of YWHAE in myeloma patients (B) and cell lines (C) with del17p vs. others. D) OS in del17p MM patients with high vs. low M protein levels undergoing bortezomib-based therapy. E) EFS and OS based on YWHAE expression.

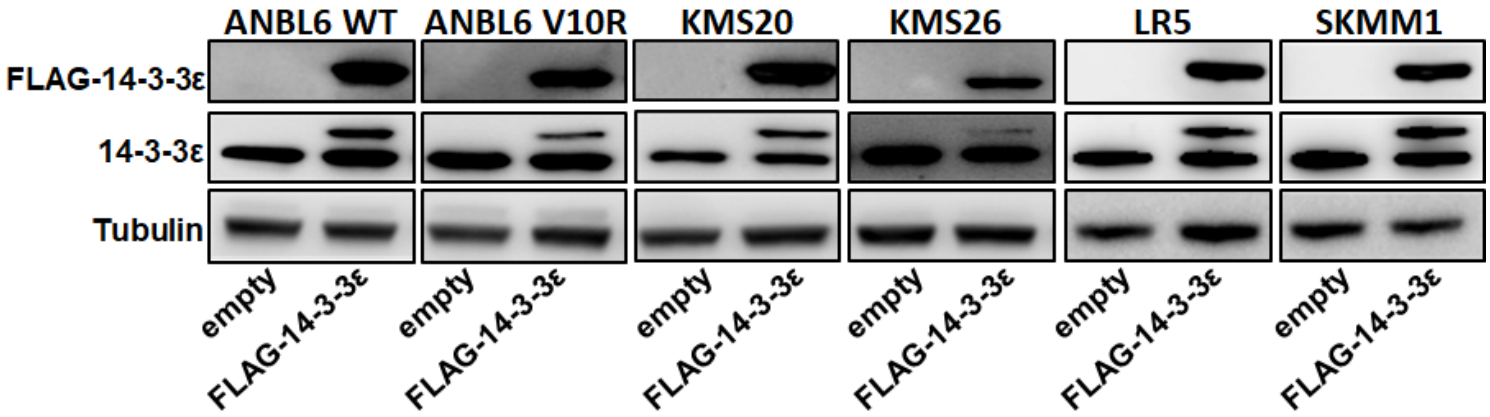


Supplementary Figure 1



Supplementary Figure 2

A



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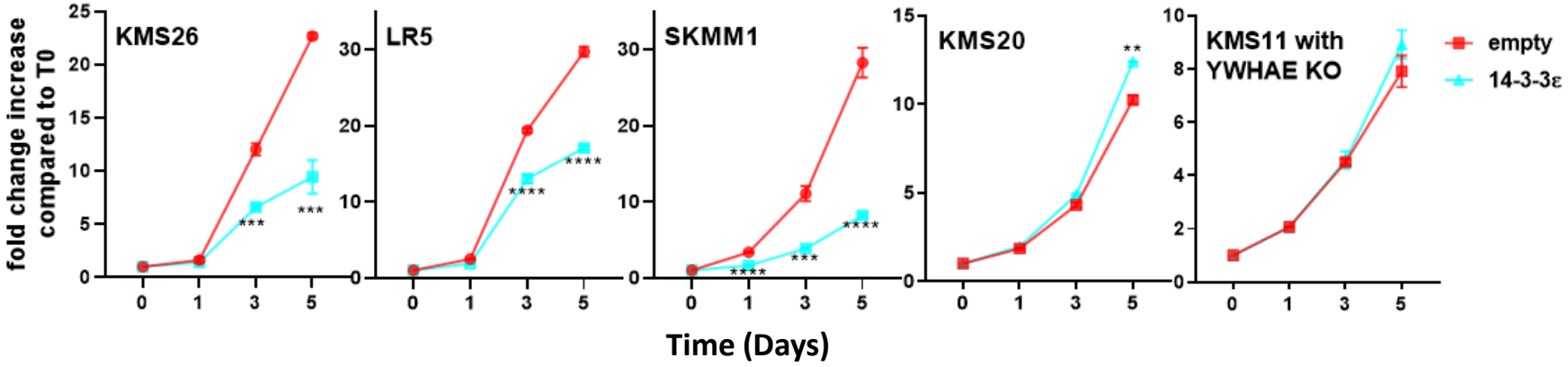
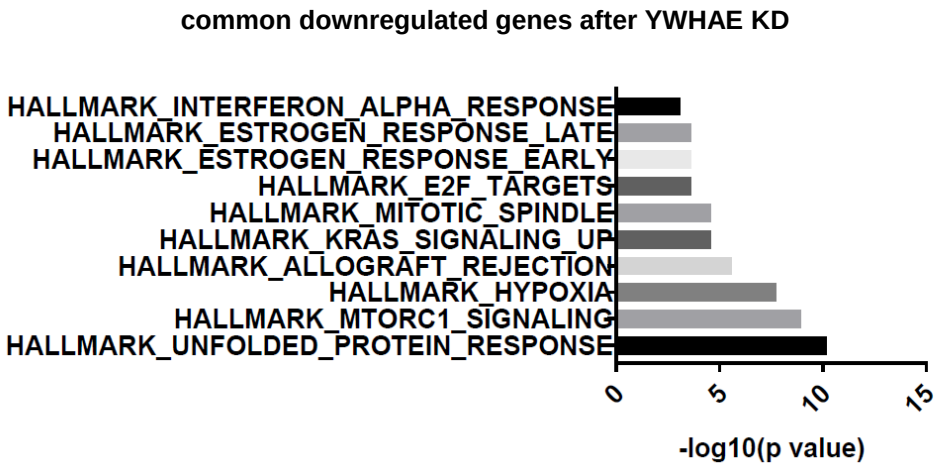


Supplementary Figure 3

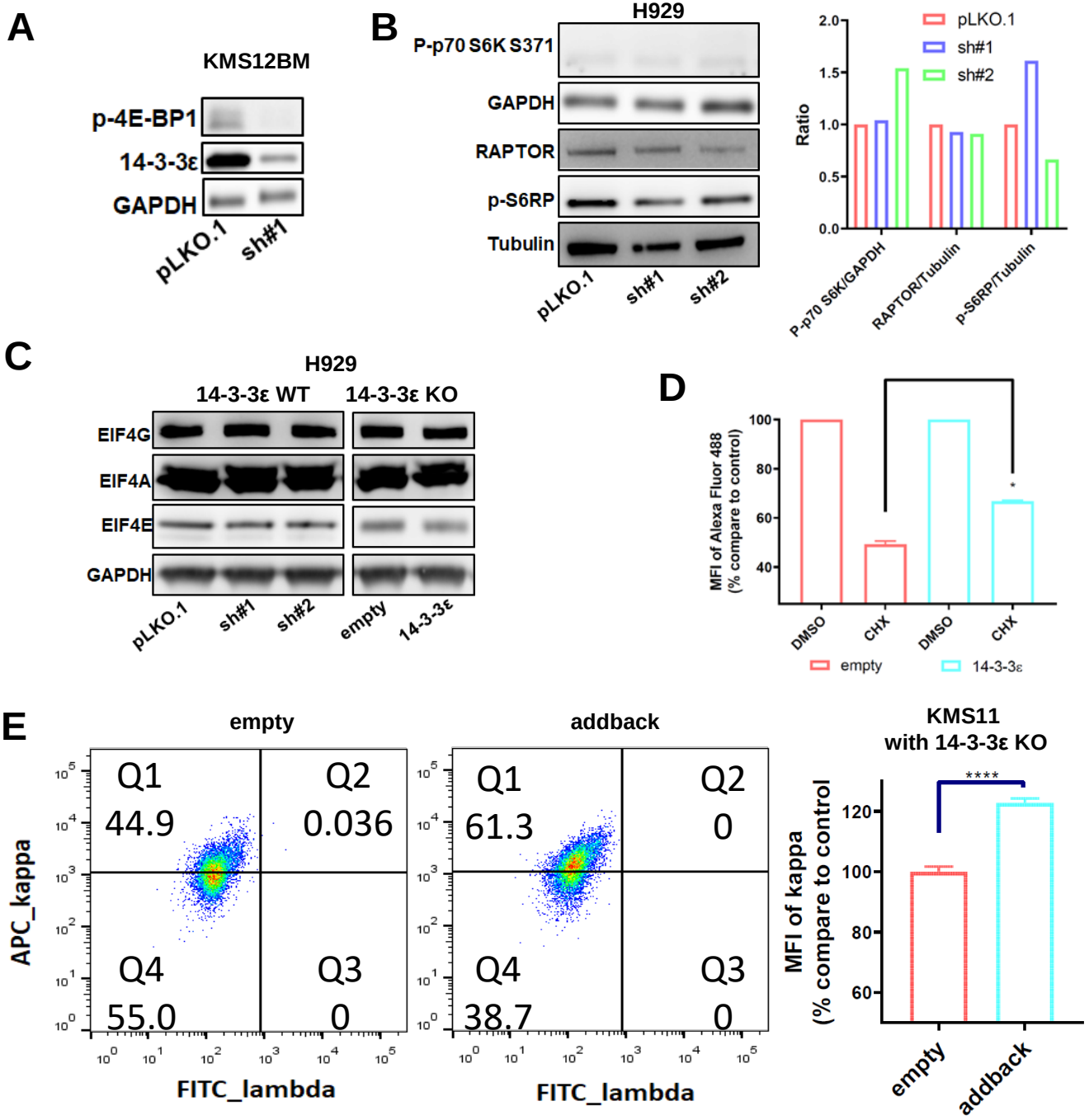
A

Gene set	Genes
PI3K AKT MTOR signaling	TIAM1 TSC2 ACTR2 ACACA SFN PRKAR2A RAF1 RPTOR MAPKAP1 AKT1S1 MTOR
MTORC1 signaling	PLK1 MCM4 PSMC4 EIF2S2 TUBG1 NUP20S ACTR2 ACACA P4HA1 ATP2A2 ABCF2 PHGDH PPA1 MTOR
UPR	KIF5B NOLC1 EIF2S1 NHP2 EXOSC5 EIF4G1 SKIV2L2 EXOC2 EIF4A3

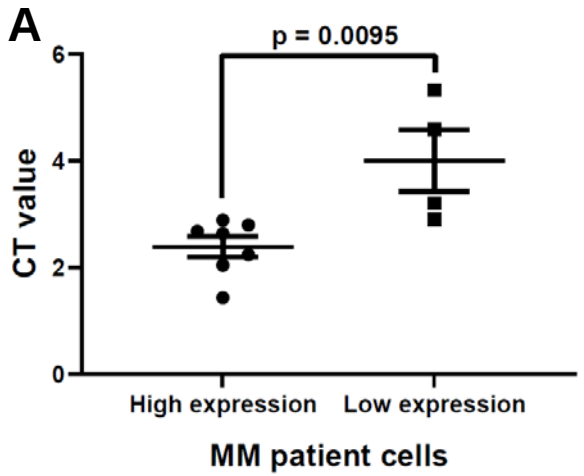
B

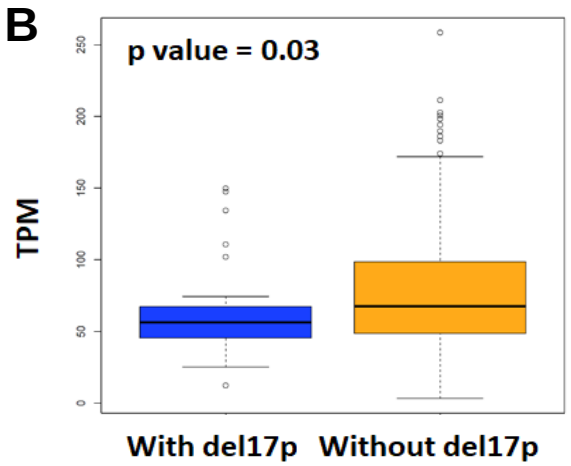


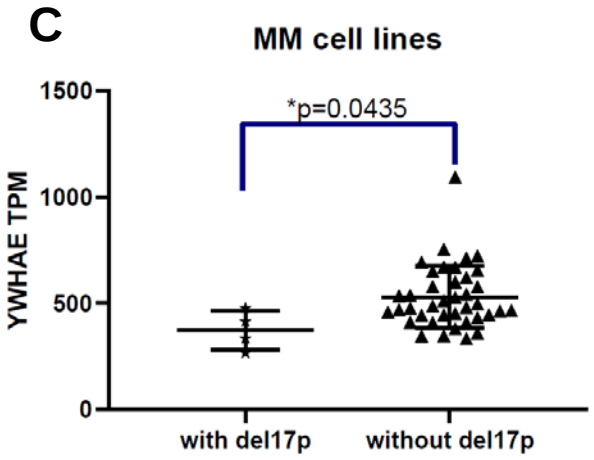
Supplementary Figure 4

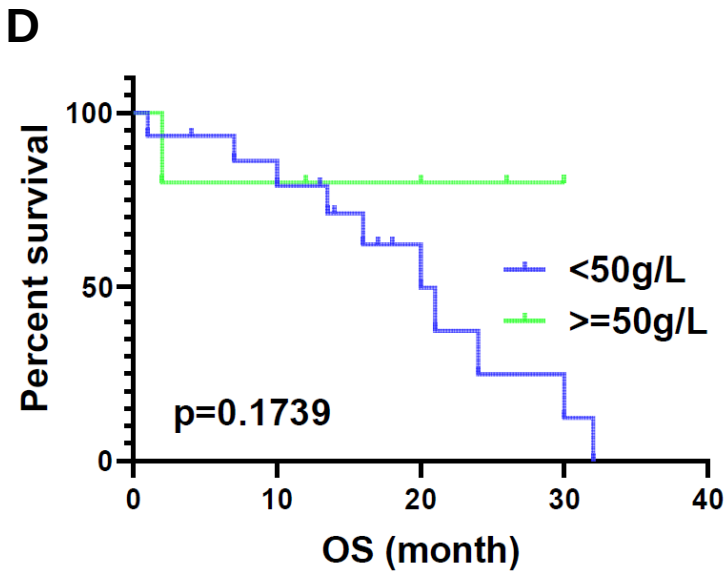


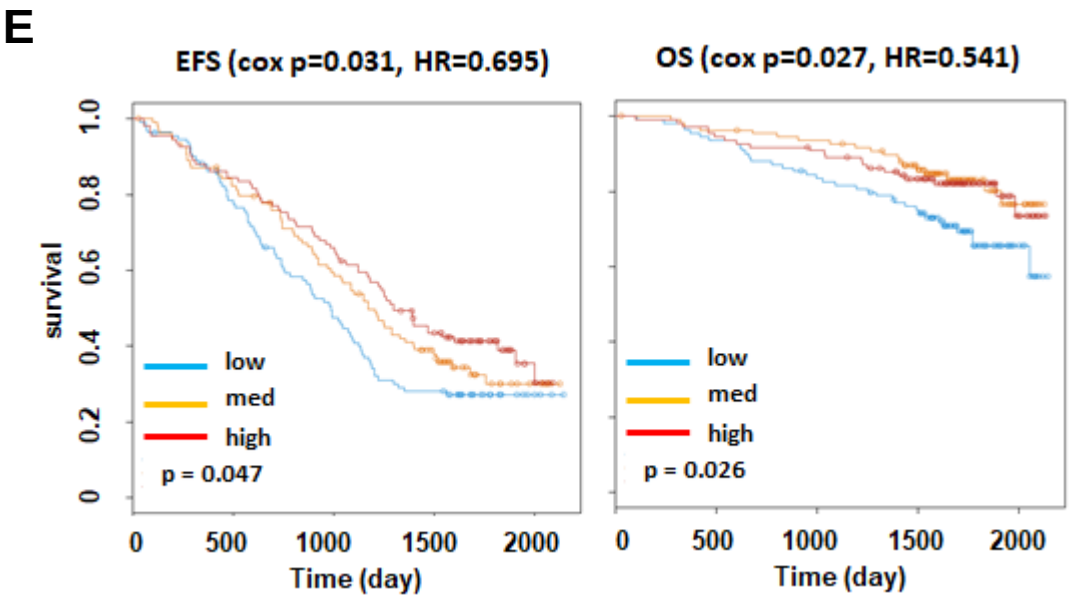
Supplementary Figure 5











Supplementary Figure 6