Supplementary Material

Table1. Antibodies used in this study

Antibody	Host	Species against	Source	Catalog number
mTOR	rabbit	human	cell signaling	2972
p-mTOR (S2448) D9C2	rabbit	human	cell signaling	5536
p-mTOR (S2481)	rabbit	human	cell signaling	2974
pAKT (S473) D9E	rabbit	human	cell signaling	4060
p4E-BP1 (S65)174A9	rabbit	human	cell signaling	9456
p-S6 Ribosomal Protein (S240/244)	rabbit	human	cell signaling	2215
Deptor	rabbit	human	cell signaling	11816
pNFκB P65 (S536)	rabbit	human	cell signaling	3033
pIκBα (S32) 14D4	rabbit	human	cell signaling	2859
IκBα (C21)	rabbit	human	Santa Cruz	Sc-371
NEDD8 (19E3)	rabbit	human	cell signaling	2754
XIAP (3B6)	rabbit	human	cell signaling	2045
BCl-XL (54H6)	rabbit	human	cell signaling	2764
BID	rabbit	human	cell signaling	2002
Caspase 3 (8G10)	rabbit	human	cell signaling	9665
Caspase 8 (1C12)	mouse	human	cell signaling	9746
Caspase 7 (C7)	mouse	human	cell signaling	9494
pH2A.X (S139) JBW301	mouse	human	Millipore	05-636
CDT-1 (D10F11)	rabbit	human	cell signaling	8064
cleaved PARP (D214) 19F4	mouse	human	cell signaling	9546
FITC conjugated CD11b (ICRF44)	mouse	human	BD	562793
CD11c APC	mouse	human	BD Pharmingen	559677
CD36 APC	mouse	human	BD Bioscience	550856
GAPDH	mouse	human	Ambion	Am4300

Table 2. Characteristics of Primary AML Cases Utilized

Case	Age	Gender	WBC	Cytogenetics	NPM1/FLT3	2º
1	73	F	237	Normal	M/+	No
2	60	M	142	Normal	M/+	No
3	61	M	441	N/A	N/A	No
4	56	F	10	Normal	M/N	Yes
5	67	M	72	-5	N/A	Yes
6	N/A	N/A	N/A	N/A	N/A	N/A
7	N/A	N/A	N/A	N/A	N/A	N/A
8	58	M	101	Inv3, 7q31	U/N	No
9	64	M	N/A	N/A	N/A	N/A
10	60	M	439K	+8	M/N	No
11	46	F	122	t(11;17)	U/N	No

Key: F=Female; M=Male; WBC=white blood count X 10(-3)/uL; Normal 46(X;X) or 46(X;Y); NPM1 (nucleophosmin) FLT3= fms-like tyrosine kinase -1 ITD (internal tandem repeat); 2º= secondary to underlying malignancy or prior myelodysplastic syndrome. N/A=not available, U=Unmutated, N=negative.

Figure S1. **mTOR inhibitor S attenuated neddylation inhibitor P induced cytotoxicity in several AML cell lines.** HL-60, KG1a, Molm-13 or THP-1 were treated with different concentrations of P with or without S for 48hours. The cell viability was determined by trypan blue staining. Live cell (trypan blue negative) and total cell number were counted by a cell counter. The cell viability was calculated by live cell number divided by total cell number times 100.

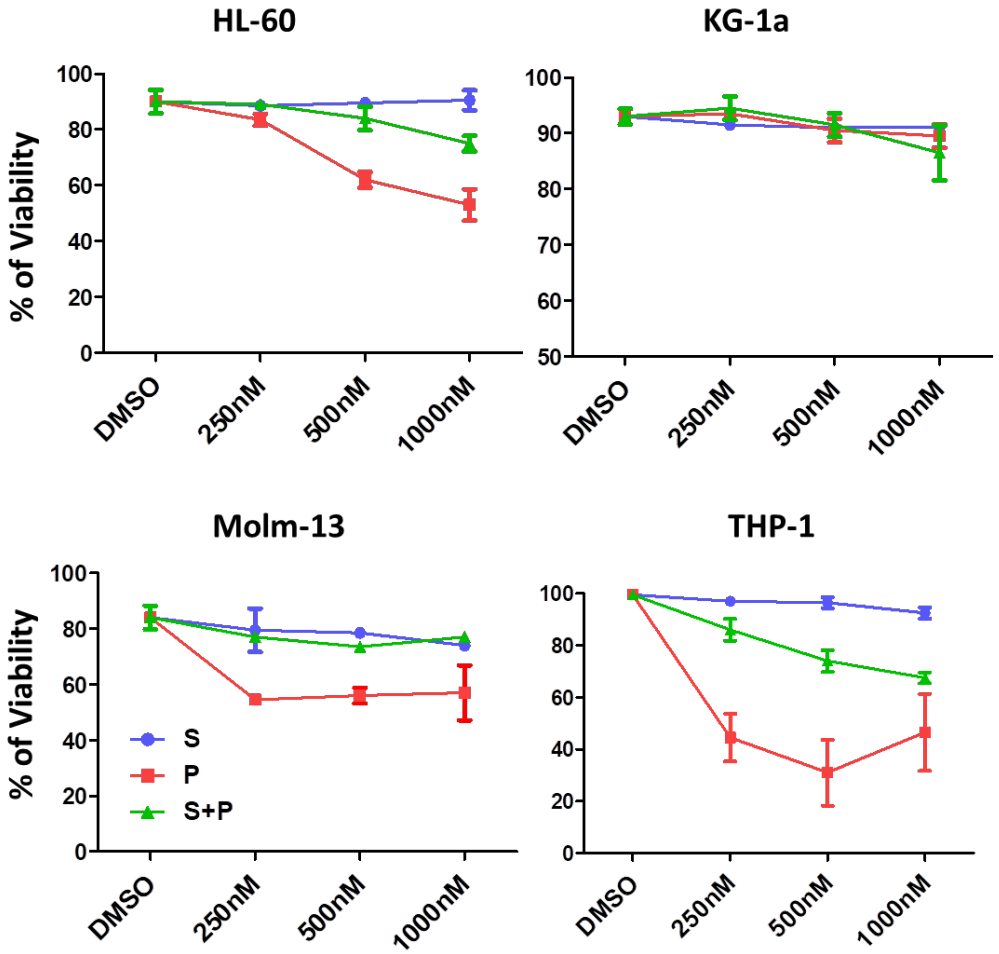

Figure S2. **Effects of S, P, or the combination on primary AML blast viability.** Primary AML blasts from apheresis samples were grown in RPMI with 10% FBS and then exposed to DMSO control, 400 nM P, 400 nM S, or the Combination (S+P) for 24 or 48 hours. Viability was assessed by Annexin V/7AAD flow cytometry analysis as described.

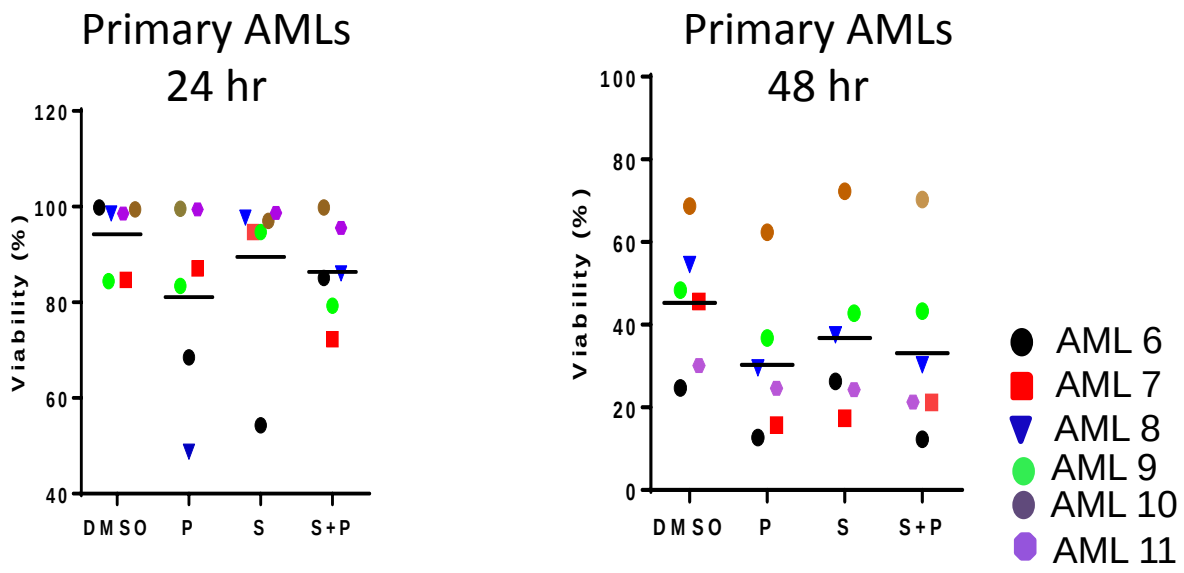

Figure S3. **Expression of CD11c and CD36 on AML cell lines in response to P or S after a 24 hour exposure**. Both drugs were used at a concentration of 400 nM. FMO=fluorescence marker only.

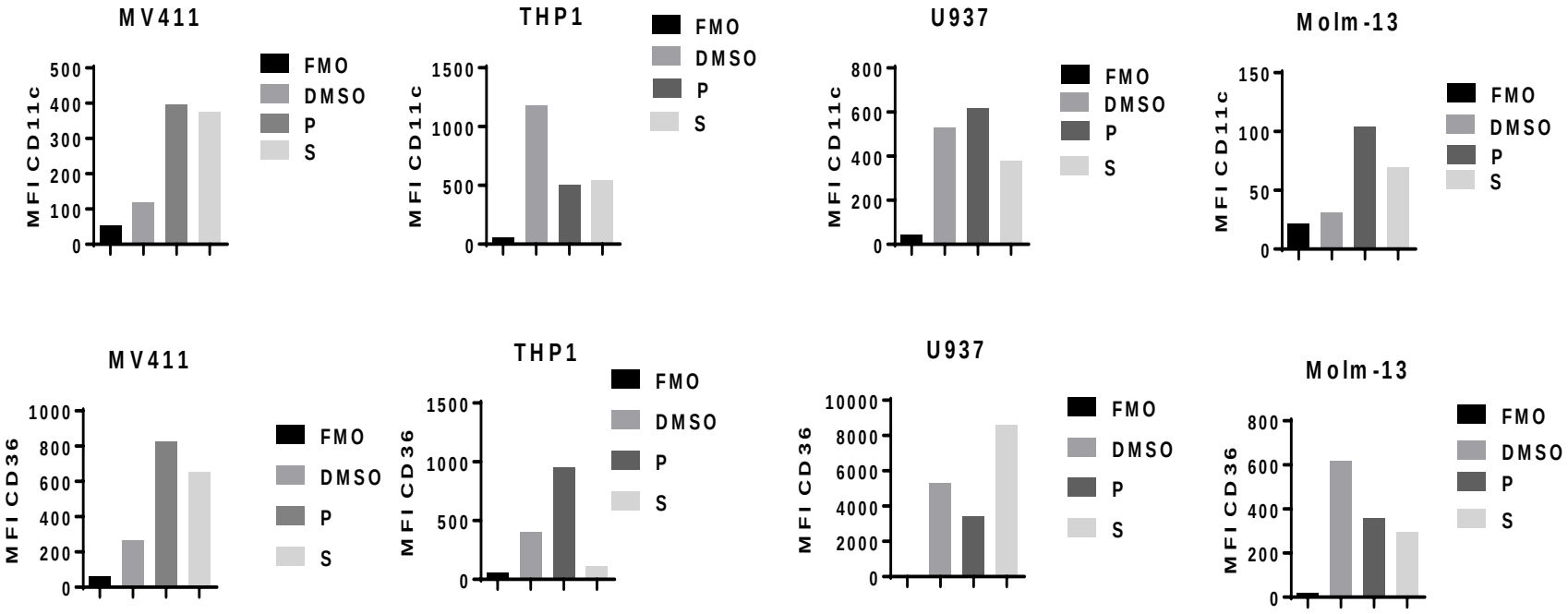

Figure S4. S significantly reduced AML cell protein levels. MV4-11 and Molm-13 were treated with 500nM S or P for 1 or 2 days, cell lysate protein levels were determined. *P<0.05, **P<0.01 compared to DMSO day1 control; ^^P<0.01, ^^^P<0.001 compared to DMSO day2 control.

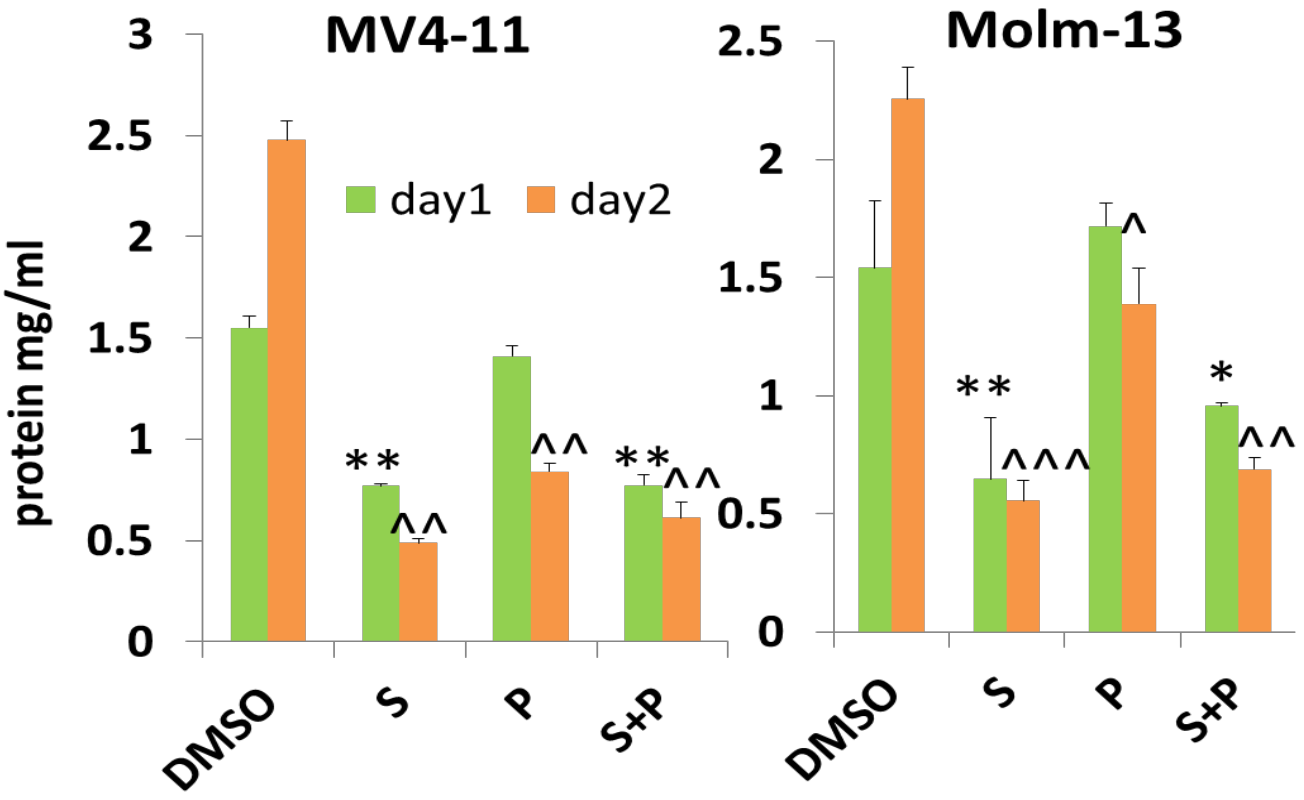

Figure S5. **mTOR shRNA decreased AML cell size.** Molm-13 cells were infected with scramble (SC) or mTOR shRNAs for 7days. Cell size was determined by flow cytometry using FSC-A parameter. *P<0.05, **P<0.01 compared to SC shRNA using student t-test.

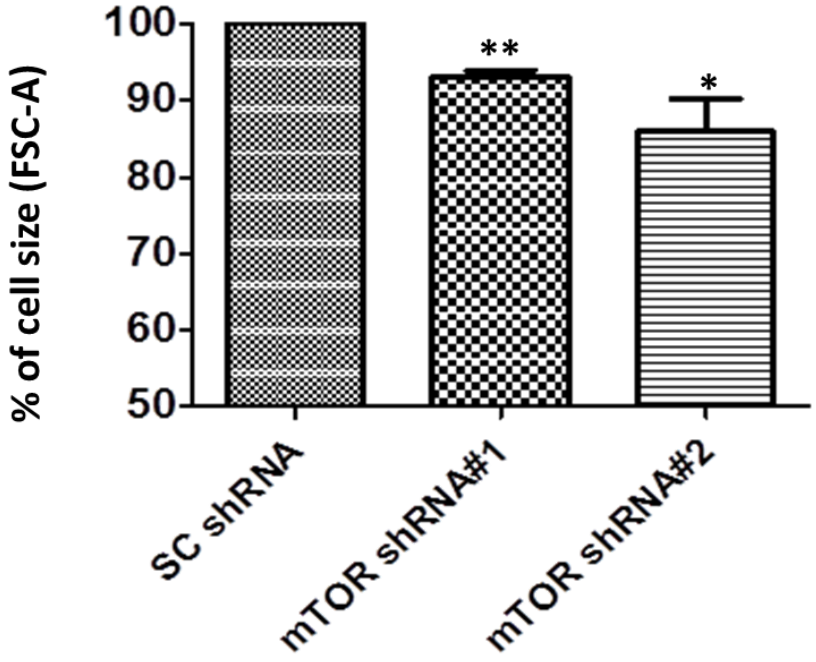

Figure S6. **mTORC1 inhibitor Rapamycin did not attenuate P induced cytotoxicity.** Molm-13 or MV4-11 cells were treated with 400nM P with or without rapamycin for 48hours. The percentage of live, apoptotic and dead cell was determined by flow cytometry annexinV apoptosis assay. Cell size was determined using FSC-A parameter.