Supplementary Materials and Methods:

TMA immunohistochemistry Commercially available antibodies against phospho-S6 ribosomal protein (pS6RP, used as a surrogate for phosphorylated S6 Kinase; Ser235/236, #2211S, Cell Signaling, Danvers, MA; 1:200; 10mM sodium citrate buffer at pH6.0 with microwave retrieval for 10min at 98°C), phospho-4EBP1 (Thr70, #9455, Cell Signaling; 1:400; 10mM sodium citrate buffer at pH6.0 with microwave retrieval for 10min at 98°C), phospho-AKT (Ser473, #9271, Cell Signaling; 1:50; Borg decloaker pH8-9 with microwave retrieval for 10min at 98°C) and PTEN (6H2.1, #M3627, DAKO, Carpinteria, CA; 1:100, Tris-HCl buffer at pH8.0 with BOND-MAX automated antigen retrieval (Leica Microsystems, Buffalo Grove, IL) were used for immunohistochemistry (IHC). Appropriate biotinylated secondary antibodies and horseradish peroxidase-labeled streptavadin (4 plus system Biocare Medical, Concord, CA) were used for immunostaining, with 3,3-diaminobenzidine serving as chromagen. Scoring was conducted by three investigators, including two soft tissue pathologists (AJL, EGD), and KBS. Samples with insufficient tumor or poor quality staining were excluded from scoring. Labeling intensity was scored as 0 (=absent/negative), 1 (=weak), 2 (=moderate) and 3 (=strong); the percentage of positively staining cells (0-100%; i.e. distribution) was estimated from the two paired TMA samples for each case.

Cell culture and reagents: Human SKLMS1 and Mes-Sa tumor cell lines were obtained from the American Type Culture Collection (ATCC). [1]Uterine leiomyosarcoma primary cultures/cell strains were isolated in our laboratory under a UTMDACC Institutional Review Board (IRB) approved protocol from surgically resected surgical specimens as previously described. These primary cultures can be reproducibly propagated for ~20-30 passages, at which time they

[1] Peng T, Zhang P, Liu J, Nguyen T, Bolshakov S, Belousov R, et al. An experimental model for the study of well-differentiated and dedifferentiated liposarcoma; deregulation of targetable tyrosine kinase receptors. Lab Invest. 2011;91:392-403.

senesce; to date none of these cell strains demonstrate immortal growth. [2]Authentication of ULMS cell strains was conducted utilizing Short Tandem Repeat DNA fingerprinting (STR) as previously described. Cells were cultured in DMEM/F-12 (with the exception of Mes-Sa cells, which were cultured in McCoy's 5A Modified Medium) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Normal smooth muscle cell primary cultures (NSMC) were obtained from ScienCell Research Laboratories (Carlsbad, CA) and propagated in Smooth Muscle Cell Medium.

Rapamycin was obtained from the UTMDACC pharmacy. MLN8237 (an investigational AURK-A inhibitor, was kindly provided by Millennium Pharmaceuticals (Cambridge, MA). For animal studies, MLN8237 was formulated in 10% 2-hydroxypropyl-β-cyclodextrin and 1% sodium bicarbonate. Commercially available antibodies were used for western blotting, IHC, or immunoflorescence to detect pAKT, AKT, Aurk-A, p4EBP1 (Thr37/46 and Thr70), 4EBP1, pS6K, S6K, pS6RP, cyclin D1, and PTEN (Cell Signaling), p21, p53, and B-actin (Santa Cruz Biotechnology, Santa Cruz, CA), CD31 (BD Pharmagen, San Diego, CA), cleaved PARP (Abcam, Cambridge, MA), and Ki67 (Thermo/Lab Vision, Kalamazoo, MI). Goat anti-rabbit IgG and goat anti-mouse IgG antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) were used as secondary antibodies. The Dead End Fluorometric TUNEL Kit (Promega, Madison, WI) was employed and used as directed for terminal deoxyribonucleotide transferase-mediated nick-end labeling (TUNEL).

[2] Xie X, Ghadimi MP, Young ED, Belousov R, Zhu QS, Liu J, et al. Combining EGFR and mTOR blockade for the treatment of epithelioid sarcoma. Clin Cancer Res. 2011;17:5901-12.

Cellular assays: [3]Western blot (WB) analyses were used to evaluate levels of protein expression and phosphorylation and were conducted as previously described; all WBs were blocked in either bovine serum albumin or milk for one hour and incubated overnight at 4°C with primary antibody (1:1000). [4]MTS assays were used to determine cell growth as previously described; drug concentrations required to inhibit cell growth by 50% (GI_{50}) were determined by interpolation of dose-response curves. [5]Colony formation assays were performed as previously described. Briefly, for pre-treatment experiments ULMS cells were pre-treated in culture dishes for 24h. Two hundred viable cells per well were re-plated and allowed to grow in normal medium for 10-14 days and then stained for 30 min at room temperature with a 5% glutaraldehyde, 0.1% crystal violet solution in 20% methanol. Images were captured digitally and colonies were counted. Alternatively, for continuous treatment, two hundred viable cells per well were first plated and then treated with drugs, at doses indicated, following the procedure as per above; drug/media was replenished every 48 hours. [6]PI staining and PI/Annexin V staining FACS analyses were conducted as previously described to evaluate cell cycle progression and rate of apoptosis, respectively.

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[3] Zhu QS, Ren W, Korchin B, Lahat G, Dicker A, Lu Y, et al. Soft tissue sarcoma cells are highly sensitive to AKT blockade: a role for p53-independent up-regulation of GADD45 alpha. Cancer Res. 2008;68:2895-903.

[4] Zou CY, Smith KD, Zhu QS, Liu J, McCutcheon IE, Slopis JM, et al. Dual targeting of AKT and mammalian target of rapamycin: a potential therapeutic approach for malignant peripheral nerve sheath tumor. Mol Cancer Ther. 2009;8:1157-68.

[5] Zhu QS, Ren W, Korchin B, Lahat G, Dicker A, Lu Y, et al. Soft tissue sarcoma cells are highly sensitive to AKT blockade: a role for p53-independent up-regulation of GADD45 alpha. Cancer Res. 2008;68:2895-903.

[6] Zhu QS, Ren W, Korchin B, Lahat G, Dicker A, Lu Y, et al. Soft tissue sarcoma cells are highly sensitive to AKT blockade: a role for p53-independent up-regulation of GADD45 alpha. Cancer Res. 2008;68:2895-903.

Xenograft immunostaining: [7]IHC and immunoflorescence studies were conducted as previously described. Ki67, TUNEL, and CD-31 counts were calculated as the average number per high power field (400x) in five separate fields of two to three independent tumors from each group. The percentage of proliferating cells as indicated by Ki67 expression was calculated by dividing the number of Ki67 positive cells by the total number of nuclei stained by Gills #3 hematoxylin per field. The percentage of apoptotic cells was calculated by dividing the number of apoptotic cells by the total number of Hoescht-stained nuclei present in each field.

[7] Zhang L, Hannay JA, Liu J, Das P, Zhan M, Nguyen T, et al. Vascular endothelial growth factor overexpression by soft tissue sarcoma cells: implications for tumor growth, metastasis, and chemoresistance. Cancer Res. 2006;66:8770-8.

Supplementary Figure 1:

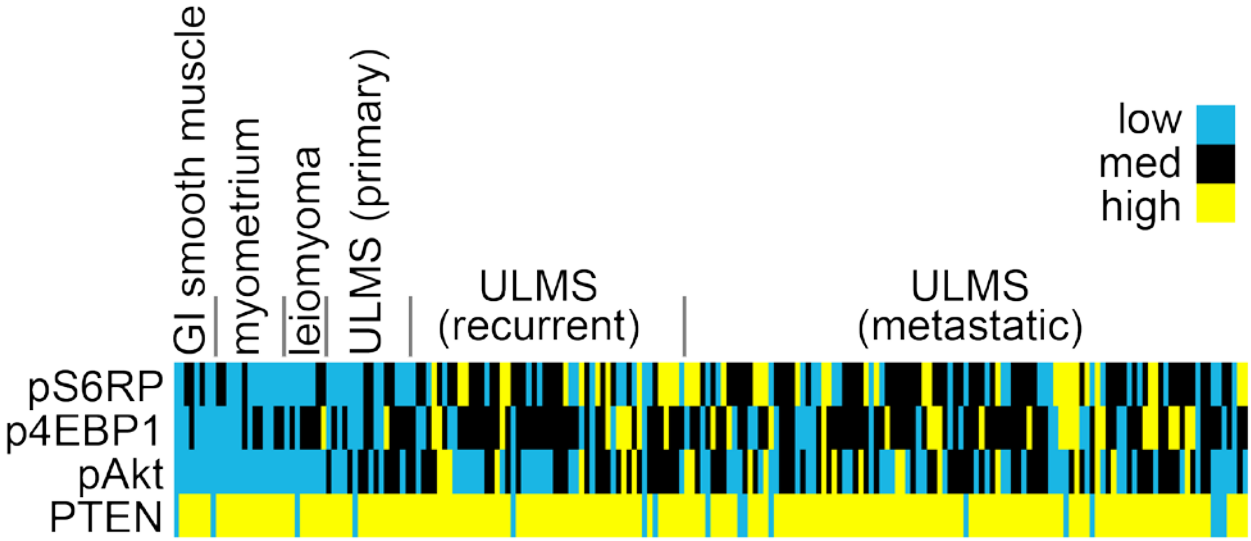
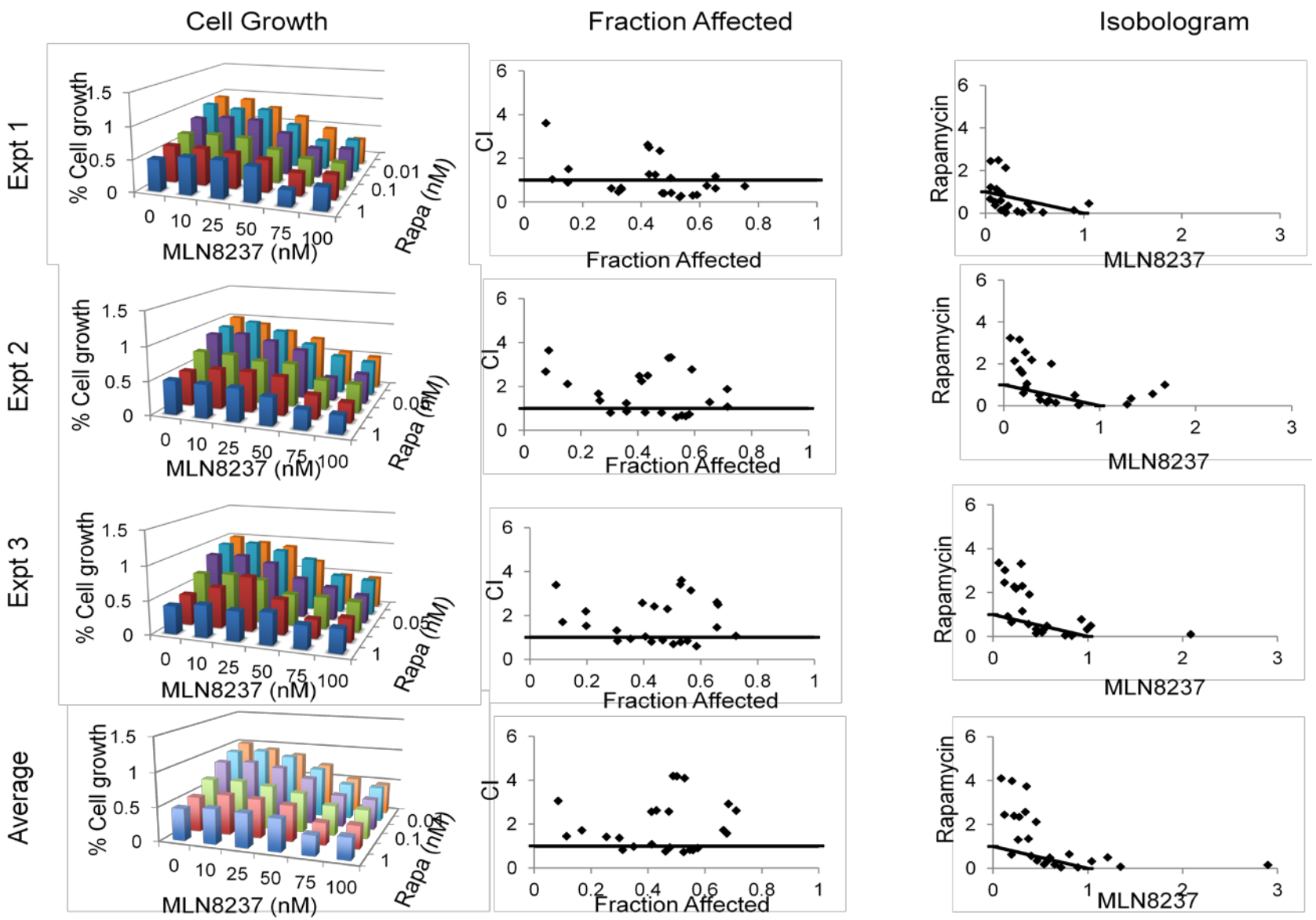
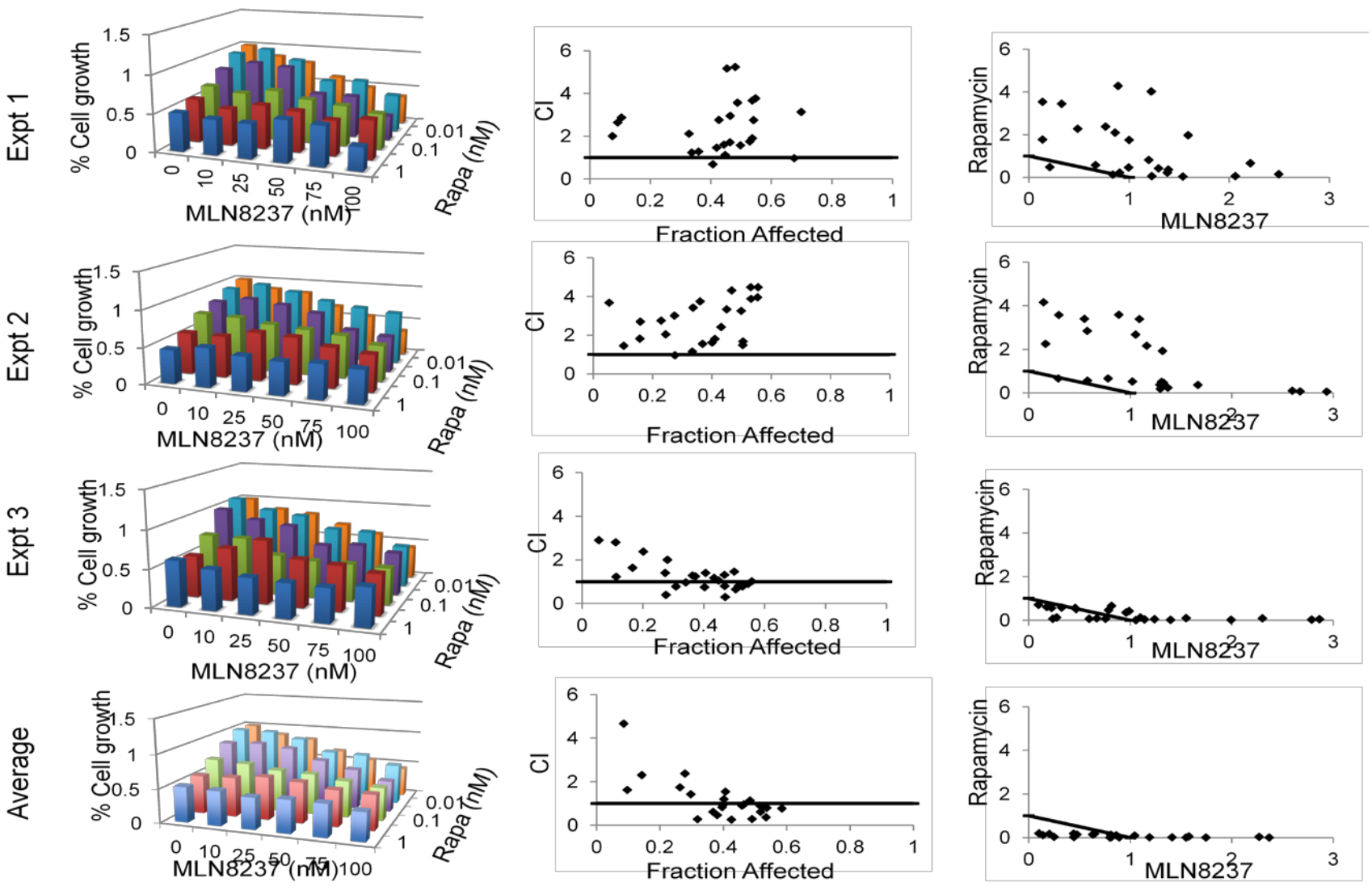


Figure S1: Heat map representation of mTOR activation associated biomarker expression levels in each of the TMA spots for which all markers were available. Of note, PTEN levels were entered as negative (“lost”) in blue and as positive (“expressed”) in yellow.

Supplementary Figure 2A. Co-Treatment



Supplementary Figure 2B. Rapamycin Pre-Treatment



Supplementary Figure 2C. MLN8237 Pre-Treatment

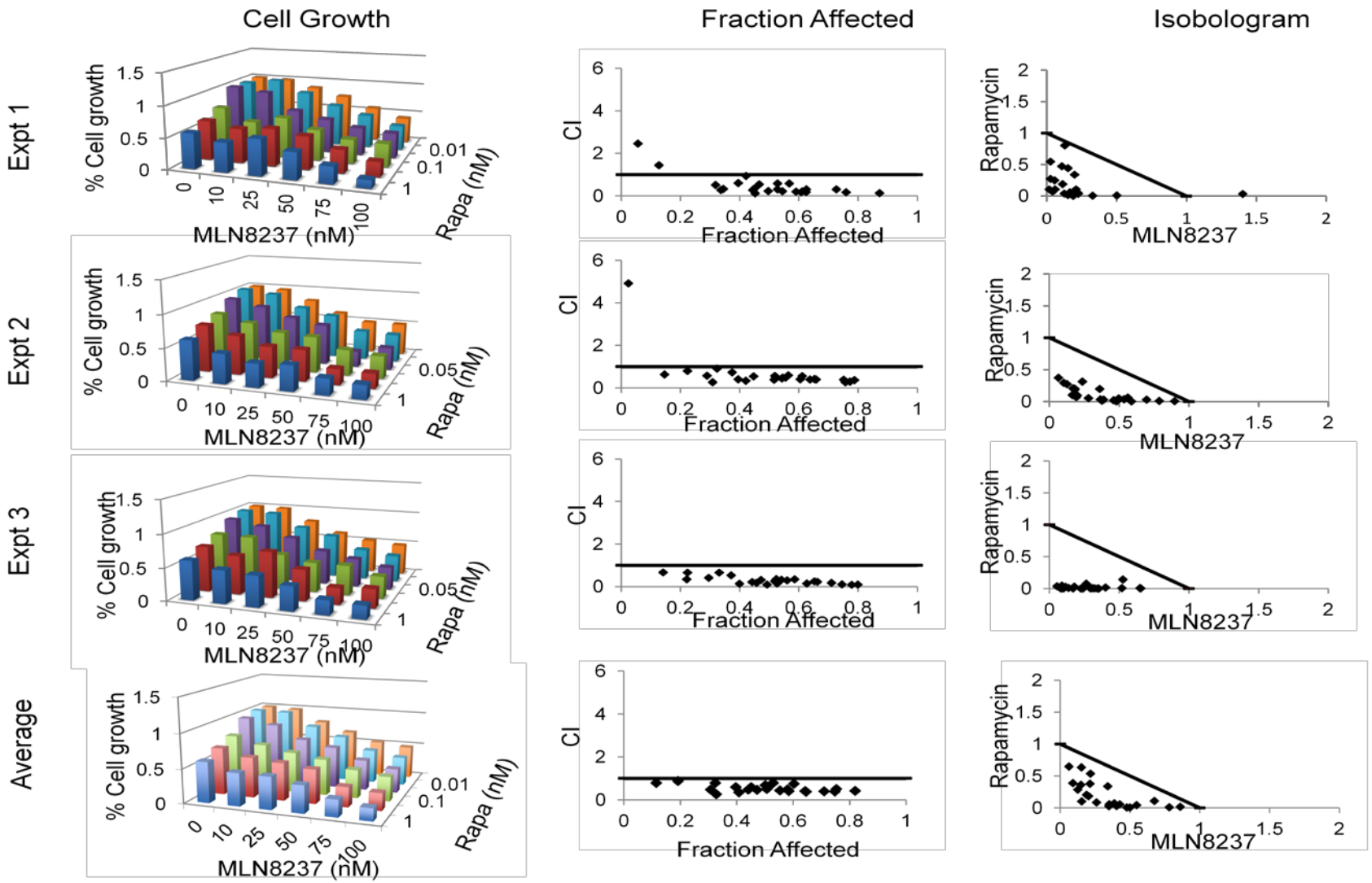


Figure S2: Results of independent studies making up the final combined Fig 5A in the text. A. drugs administered together. B. Rapamycin administered first. C. MLN8237 administered first.

Supplementary Figure 3:

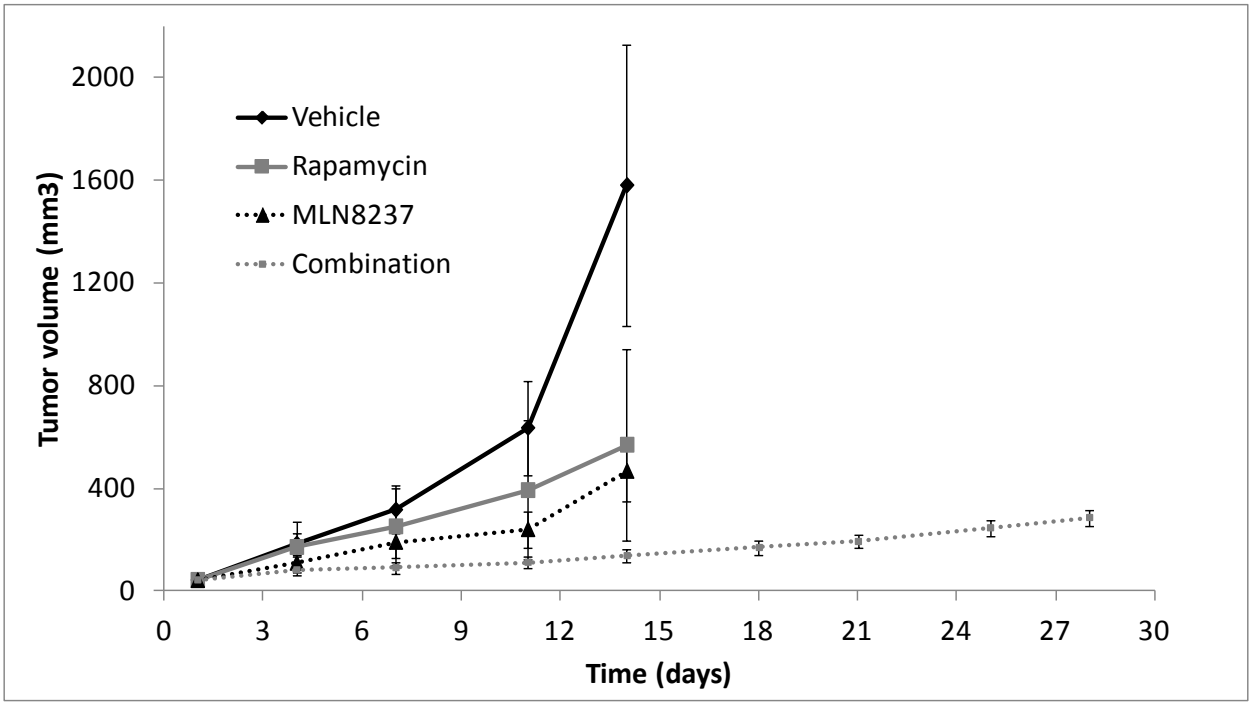


Fig S3: The animal experiment depicted in Fig 6 assessing the impact of combined therapy (Rapamycin: 3.75mg/kg/d, five days a week and MLN8237:15mg/kg twice daily, every day) was repeated using SKLMS1 xenografts growing in hairless SCID mice. Average tumor volumes at the initiation of the study were control group: 43.5mm^3±20.1 rapamycin group: 43.6mm^3±35.6, MLN8237: 42.5mm^3±22.2, and combination: 43.7mm^3±18. No major side effects or discomforts were noted. Both compounds and their combination delayed tumor growth over time in a statistically significant manner (two-way ANOVA<0.01). Mice in control, rapamycin, and MLN8237 groups were euthanized when tumor size of control groups reached an average of >15mm in larger dimension (volumes: control: 1580mm^3±548, rapamycin: 570mm^3±373, MLN8237: 468mm^3±117.5). Combination treatment group average volume at this time was 138mm^3±25.5. Treatment was continued for this later group for an additional two week period demonstrating slight additional growth (volume at euthanasia: 286.6mm^3±83). Validating the results of the first study, combination therapy resulted in superior anti-ULMS effects.

Supplementary Table 1 (Table S1): biomarker correlation between disease status and tumor status**.**

A.	**Control**				**Tumor**		
	Smooth Muscle		Leiomyoma		ULMS		
Marker	Total n=	Moderate-High Intensity[#] (%)	Total n=	Moderate-High Intensity[#] (%)	Total n=	Moderate-High Intensity[#] (%)	***P ****
pS6RP intensity	25	7 (28%)	8	2 (25%)	200	145 (73%)	**<0.0001**
cyt. p4EBP1 intensity	23	10 (43%)	7	2 (29%)	193	150 (78%)	**<0.0001**
nuc. p4EBP1 intensity	24	5 (22%)	7	3 (43%)	192	117 (61)	**0.0005**
pAkt intensity	25	0 (0%)	7	0 (0%)	187	110 (59%)	**<0.0001**
PTEN loss[@]	25	2 (8%)	8	1 (13%)	203	15 (7%)	0.72

B.	**Primary ULMS**		**Recurrent ULMS**		**Metastatic ULMS**		
Marker	Total n=	Moderate-High Intensity[#] (%)	Total n=	Moderate-High Intensity[#] (%)	Total n=	Moderate-High Intensity[#] (%)	***P *****
pS6RP intensity	18	6 (33%)	62	47 (76%)	120	92 (77%)	**0.0005**
cyt. p4EBP1 intensity	16	9 (56%)	59	50 (85%)	118	91 (77%)	**0.05**
nuc. p4EBP1 intensity	16	4 (25%)	59	39 (66%)	118	74 (63%)	**0.0088**
pAkt intensity	16	9 (56%)	58	35 (60%)	113	66 (58%)	0.95
PTEN loss[@]	18	1 (6%)	64	4 (6%)	121	10 (8%)	0.91

All p values were calculated by chi-squared test, except where 50% of the cells had expected counts less than 5%, in which case Fisher's exact test was applied.
Total n= indicates the number of evaluable samples in each dataset
**P compares control versus tumor tissues*
*** P compares primary versus advanced (recurrent and metastatic) disease*
[#]moderate to high intensity is defined as having intensity 2 or 3 on a scale of 0-3 where 0=not present, 1=weakly present, 2=moderately present, 3=highly present
[@]loss is defined as a score of 0 on a scale of 0-3 (defined in[#]) and indicates complete protein loss
Statistically significant correlations are marked in bold text
P-values are one-sided

Supplementary Table 2 (Table S2): Correlation between mTOR associated biomarkers' expressions (Spearman Rank Correlation)

	pS6RP	Cytoplasmic p4EBP1	Nuclear p4EBP1	pAKT	PTEN
pS6RP		**r=0.213 *p=0.002* (n=186)**	**r=0.193 *p=0.004* (n=186)**	**r=0.145 *p=0.027* (n=180)**	r=0.056 *p=0.217* (n=197)
Cytoplasmic p4EBP1	**r=0.213 *p=0.002* (n=186)**		**r=0.416 *p<0.001* (n=193)**	**r=0.247 *p<0.001* (n=185)**	r=0.053 *p=0.236* (n=189)
Nuclear p4EPB1	**r=0.193 *p=0.004* (n=186)**	**r=0.416 (p<0.001, n=193)**		**r=0.216 *p=0.002* (n=185)**	r=0.036 *p=0.310* (n=189)
pAKT	**r=0.145 *p=0.026* (n=180)**	**r=0.247 *p<0.001* (n=185)**	**r=0.216 *p=0.002* (n=185)**		r=0.099 *p=0.091* (n=183)
PTEN	r=0.056 *p=0.217* (n=197)	r=0.053 *p=0.236* (n=189)	r=0.036 *p=0.310* (n=189)	r=0.099 *p=0.091* (n=183)	

Supplementary Table 3 (Table S3): Univariable Cox proportional hazard models for biomarker expression correlation with ULMS patients' outcome and multivariable analysis for DSS

	Disease-Specific Survival (univariable)		Disease-Specific Survival (multivariable)*	
Marker	***P***	**Hazard Ratio (95% CI)**	***P***	**hazard ratio (95% CI)**
pS6RP intensity	0.1759	-	-	-
pS6RP distribution	0.8796	-	-	-
cyt. p4EBP1 intensity	**0.0856**	1.07 (0.93-3.13)	**0.0089**	3.55 (1.37-9.18)
cyt. p4EBP1 distribution	**0.0793**	1.03 (1.00-1.07)	**0.0288**	1.05 (1.01-1.11)
nuc. p4EBP1 intensity	**0.0750**	1.57 (0.96-2.59)	**0.0162**	2.27 (1.16-4.42)
nuc. p4EBP1 distribution	**0.0494**	1.03 (1.00-1.06)	**0.0138**	1.06 (1.01-1.11)
pAkt intensity	0.9899	-	-	-
pAkt distribution	0.4645	-	-	-

p-values bolded indicate statistically significant ($p<0.05$) values

Bolded and greyed p values indicate values <0.1 values (included in multivariable analysis for DSS)

*multivariable analysis evaluated a model that included marker expression and the clinical parameters: tumor size, surgical margins (R0, R1, R2), recurrence status, and age.

Supplementary Table 4 (Table S4): Averaged fraction affected (FA), combination index (CI), averages and standard deviation values for synergy experiments.

MLN8237 (nM)	Rapamycin (nM)	Combination treatment				Rapamycin pre-treatment				MLN8237 pre-treatment			
		Fa	Cl	Avg	Stdev	Fa	Cl	Avg	Stdev	Fa	Cl	Avg	Stdev
10	0.01	0.022	6.088	0.978	0.066	$1x10^{-11}$	$5.4x10^{12}$	1.003	0.051	$1x10^{-9}$	$8.7x10^{8}$	1.008	0.048
	0.05	0.113	1.451	0.887	0.036	0.097	1.630	0.903	0.020	0.115	0.783	0.885	0.051
	0.1	0.311	0.825	0.689	0.009	0.319	0.283	0.680	0.075	0.328	0.255	0.672	0.117
	0.5	0.412	2.566	0.590	0.014	0.426	0.261	0.582	0.103	0.441	0.477	0.587	0.026
	1	0.488	4.186	0.512	0.054	0.490	0.298	0.510	0.040	0.521	0.708	0.479	0.026
25	0.01	0.084	3.054	0.916	0.009	0.086	4.668	0.914	0.029	0.192	0.875	0.808	0.056
	0.05	0.167	1.709	0.833	0.026	0.143	2.307	0.857	0.033	0.308	0.479	0.692	0.026
	0.1	0.349	0.973	0.651	0.015	0.380	0.472	0.620	0.078	0.408	0.344	0.592	0.057
	0.5	0.430	2.621	0.570	0.022	0.368	0.620	0.632	0.051	0.452	0.591	0.548	0.061
	1	0.501	4.180	0.499	0.073	0.535	0.373	0.465	0.011	0.529	0.785	0.471	0.108
50	0.01	0.253	1.420	0.747	0.052	0.264	1.750	0.737	0.101	0.324	0.790	0.676	0.006
	0.05	0.299	1.366	0.701	0.035	0.298	1.431	0.702	0.131	0.397	0.590	0.603	0.041
	0.1	0.412	1.085	0.588	0.056	0.397	0.842	0.603	0.026	0.472	0.460	0.415	0.038
	0.5	0.473	2.576	0.527	0.034	0.399	0.939	0.601	0.056	0.502	0.678	0.499	0.031
	1	0.529	4.090	0.471	0.064	0.518	0.623	0.482	0.056	0.602	0.750	0.398	0.031
75	0.01	0.461	0.760	0.539	0.060	0.279	2.374	0.721	0.049	0.506	0.506	0.494	0.050
	0.05	0.525	0.728	0.475	0.019	0.402	1.202	0.598	0.040	0.554	0.433	0.446	0.008
	0.1	0.558	0.814	0.442	0.025	0.459	0.910	0.541	0.048	0.585	0.405	0.415	0.038
	0.5	0.677	1.571	0.323	0.041	0.465	0.968	0.535	0.069	0.710	0.392	0.290	0.073
	1	0.710	2.613	0.290	0.047	0.523	0.824	0.477	0.040	0.752	0.507	0.247	0.026
100	0.01	0.476	0.937	0.524	0.009	0.407	1.553	0.593	0.118	0.580	0.481	0.420	0.014
	0.05	0.547	0.829	0.453	0.042	0.538	0.809	0.463	0.124	0.646	0.373	0.354	0.018
	0.1	0.575	0.909	0.425	0.019	0.516	0.911	0.484	0.012	0.642	0.403	0.358	0.027
	0.5	0.665	1.719	0.335	0.046	0.485	1.144	0.516	0.015	0.748	0.385	0.252	0.033
	1	0.683	2.914	0.317	0.048	0.585	0.776	0.415	0.102	0.820	0.411	0.280	0.046