Supplemental Data

Prolonged Rapamycin Treatment Inhibits mTORC2 Assembly and Akt/PKB

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Supplemental Experimental Procedures

Rapamycin Treatment of Mice and Organ Harvest

Rapamycin (1 mg) was dissolved in 20 μl of ethanol, which was then diluted with Ringer's saline solution to a final concentration of 1 mg/ml directly before use. Three-month-old male C57BL/6NTac (Taconic) mice were administered daily intraperitioneal injections of 10 mg/kg rapamycin or the drug vehicle for 7 days. Mice were then euthanized with $C0_2$, and organs were harvested into RIPA buffer and homogenized with mechanical disruption followed by sonication. Lysates from vehicle- and rapamycin-treated organ pairs were normalized for protein content and analyzed by immunoblotting. The vehicle- and rapamycin-treated mice ate similar amounts during the 7 day treatment period and at necropsy all mice had evidence of processed food in their stomachs and small intestines. Control experiments using phospho-S6 as a marker of the effectiveness of rapamycin reveals that the drug penetrates all major tissues. The experiment was repeated twice with similar results.

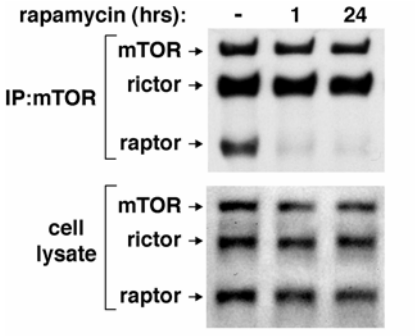

Figure S1. Prolonged In Vitro Incubation of mTORC1 and mTORC2 with Rapamycin Leads to Disruption of the Raptor-mTOR but Not Rictor-mTOR Interaction

A HeLa cell lysate was prepared with a CHAPS-based lysis buffer and divided into three equal portions. One was incubated with 100 nM rapamycin for 1 hr, another with rapamycin for 24 hr, and the third with the drug vehicle for 1 hr. mTOR immunoprecipitates were then prepared and analyzed by immunoblotting for the levels of mTOR, rictor, and raptor.

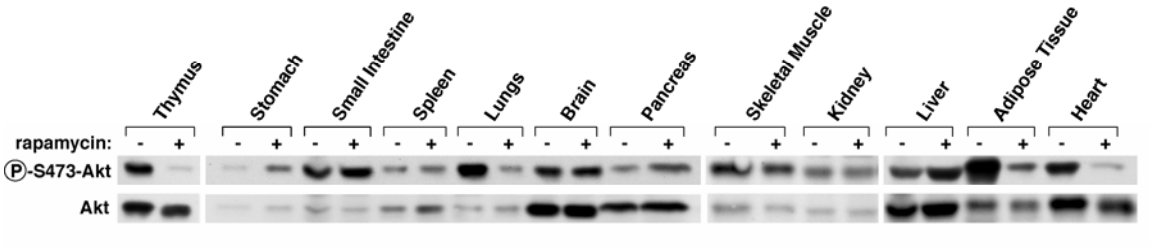

Figure S2. Rapamycin Inhibits Akt/PKB Phosphorylation In Vivo

Mice were treated for 1 week with daily intraperitoneal injections of rapamycin or the drug vehicle. Tissues were harvested as described in the Experimental Procedures, and Akt/PKB S473 phosphorylation and protein levels were monitored by immunoblotting. Reminiscent of the behavior of many cell lines (Figure 2), several tissues, notably the stomach and liver, showed rapamycin-induced increases in Akt/PKB phosphorylation.

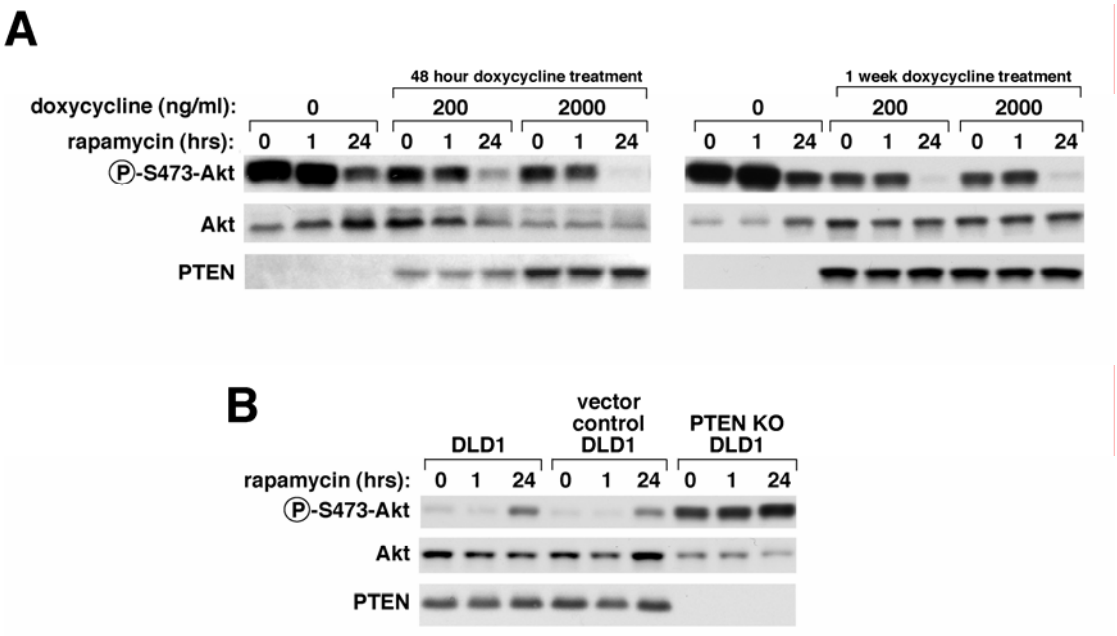

Figure S3. PTEN Loss Is Neither Necessary nor Sufficient to Confer Rapamycin-Sensitive Akt/PKB Phosphorylation to a Cell Line

(A) PTEN-null Jurkat cells having a doxycycline-inducible PTEN were cultured for 24 hr (left) or 1 week (right) in the indicated concentrations of doxycyline. The cells were then treated with 100 nM rapamycin for the indicated times and analyzed by immunoblotting for the levels of phospho-S473 Akt/PKB, Akt/PKB, and PTEN.

(B) Parental DLD1 cells, DLD1 cells having a stably integrated vector (vector control DLD1), and DLD1 cells null for PTEN were treated with 100 nM rapamycin for the indicated times and analyzed by immunoblotting for the levels of phospho-S473 Akt/PKB, Akt/PKB, and PTEN. DLD1 cells are in the class of cells that increase Akt/PKB phosphorylation with rapamycin treatment.

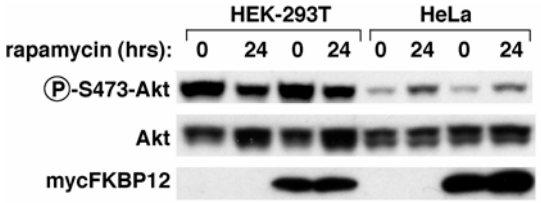

Figure S4. Stable Overexpression of FKBP12 Does Not Confer Rapamycin-Sensitive Akt/PKB Phosphorylation

HEK-293T or HeLa cells stably expressing myc-FKBP12 or transduced with the empty vector were treated with 100 nM rapamycin or drug vehicle for 24 hours and analyzed by immunoblotting for the indicated proteins and phosphorylation states.

Supplemental Table S1.

		Effect of 24 hr rapamycin treatment on Akt/PKB phosphorylation				
Tissue of Origin/Cancer Type	Cell Line Name	Strong Inhibition	Partial Inhibition	None or Increase	PTEN Status*	Species
Lymphoma/Leukemia	**Jurkat**	√			null	human
	BJAB	√			null	human
	SKW3		√			human
	U937	√			null	human
	WEHI			√		mouse
	K562			√	null	human
Breast Cancer	**MDA-MB-231**			√		human
	MDA-MB-468			√	null	human
Multiple Myeloma	**OPM2**	√			null	human
	Δ47			√	null	human
Prostate Cancer	**PC3**	√			null	human
	LNCaP			√	null	human
Colorectal Cancer	**HT29**			√		human
	CACO2			√		human
	SW480			√		human
Endometrial Cancer	**Ishikawa**			√	null	human
Cervical Cancer	**HeLa S3**			√		human
	HeLa			√		human
Osteosarcoma	**U2OS**			√		human
Hepatic Cancer	**HepG2**	√				human
Melanoma/Epithelial	**UACC-903**		√		null	human
	Mel-STRG			√		human
	A375			√		human
	HMLE			√		human
Rhabdomyosarcoma	**Kym-1**			√		human
	Rd88SC.10			√		human
	rh30			√		human
Glioblastoma	**u87**		√		null	human
	827			√	null	human
Lung Cancer	**A549**			√		human
	H460			√		human
Renal Carcinoma	**786-0**			√	null	human
Kidney transformed	**HEK-293T**		√			human
Fibroblasts	**MEFs (p53 -/-)**		√		wild-type	mouse
	BJ Fibroblasts			√	wild-type	human
Skeletal Muscle	**c2c12 myoblasts**	√			wild-type	mouse
Smooth Muscle Cells	**rSMC (primary)**		√		wild-type	rat
Endothelial Cells	**HUVEC (primary)**	√			wild-type	human
Adipocytes	**3t3L1 differentiated**		√		wild-type	mouse

** PTEN status is only noted if evidence is available from the literature. Blank boxes indicate that the status is unknown to us.*

Table S1. Survey of 33 Cancer/Transformed and Six Primary Cell Lines for Rapamycin Sensitivity of Akt/PKB Phosphorylation

Cells were treated with 100 nM rapamycin for 24 hours and processed as in Figure 2. PTEN status was determined from the literature and is indicated only where status is certain. Empty boxes indicate that PTEN status is unknown to us. Using immunoblotting for PTEN we confirmed unpublished references found on the internet that claim that BJAB cells are null for PTEN.