Supplementary Figure and Table Legends

Supplementary Figure 1 – mTOR pathway mutations

The canonical mTOR pathway is depicted. The width of each represented gene indicates the length of its amino acid sequence and the intensity of color reflects the percentage of recurrent mutations observed for that gene in our database. Green and red color-coding reflects positive/negative pathway components, respectively.

Supplementary Figure 2 – S2215Y mTOR activates mTORC1 signaling similar to WT and mutant PI3K-p110α

A – HEK-293T cells were co-transfected with HA-GST-S6K1 and cDNAs for WT mTOR, S2215Y mTOR, WT, E545K, or H1047R PI3K-p110α cDNAs in expression vectors followed by whole cell lysis 48 hours after transfection. The lysates were then immunoblotted for the indicated proteins and the phosphorylation state of S6K1.

B – Densitometry of pS6K1 vs. HA-GST-S6K1 from A is shown.

Supplementary Figure 3 – Non-recurrent *MTOR* mutations confer different degrees of mTORC1 activation

A-C, All annotated amino acid substitutions from cancer samples in codons C1483, S2215, and I2500 were tested for mTORC1 signaling. HEK-293T cells were co-transfected with HA-GST-S6K1 and either mTOR WT or mutant cDNAs in expression vectors followed by glutathione precipitation 48 hours after transfection. The lysates and precipitates were then immunoblotted for the indicated proteins and the phosphorylation state of S6K1. The number of samples with the given mutation from our database is indicated after the label on the immunoblot.

D – Pie charts indicating the percentage of samples contributing to the C1483 and S2215 clusters are shown.

Supplementary Figure 4 – mTOR mutants alter Deptor binding and nutrient deprivation, but not sensitivity to mTOR inhibitors

A – Exogenously expressed WT and mutant mTOR co-immunoprecipitate equally well endogenous Raptor and Rictor, but have reduced Deptor binding. HEK-293T cells were co-transfected with mTOR WT or mutant cDNAs in expression vectors, lysates were prepared 48 hours after transfection, and the anti-FLAG immunoprecipitates or lysates were immunoblotted for the indicated proteins.

B – TREX cells express WT and mutant mTOR proteins. TREX cell lines were generated as indicated in the methods, treated with doxycycline for ten days, followed by whole cell lysis. The lysates were then immunoblotted for the indicated proteins.

C and D – TREX cell lines are sensitive to pharmacological mTOR inhibitors. TREX cell lines expressing WT or mutant mTOR were subjected to various pharmacological mTOR inhibitors for 60 minutes, followed by whole cell lysis. The lysates were then immunoblotted for the indicated proteins.

E and F – TREX cells expressing mTOR A1459P, S2215Y, I2500F, or R2505P are resistant to nutrient deprivation. TREX cell lines expressing WT or mutant mTOR were starved of amino acids or glucose for 60 minutes followed by re-feeding with the appropriate nutrient, followed by whole cell lysis. The lysates were then immunoblotted for the indicated proteins.

Supplementary Figure 5 – mTOR R1208C, V2006L, R2152C, and R2430M fail to confer increased S6K1 phosphorylation. HEK-293T cells were co-transfected with HA-GST-S6K1 and mTOR WT or mutant cDNAs in expression vectors followed by whole cell lysis 48 hours after

transfection. The lysates were then immunoblotted for the indicated proteins. The R1208C mutation is a SNP found in the HEC59 cell line.

Supplementary Figure 6 – Recurrent mTOR mutations mapped onto the crystal structure of mTOR. The three-dimensional positions of several of the most recurrent mutations in mTOR are shown.

Supplementary Table 1 – All mutations from the cBIO and COSMIC databases in mTOR pathway genes, excluding *MTOR*, are listed.

Supplementary Table 2 – All *MTOR* mutations from a literature search and the cBIO, COSMIC, and ICGC databases are listed.

Supplementary Table 3 – Homologous mutations from S. pombe *tor2* in H. sapiens *MTOR* are listed.

Supplementary Table 4 – All activating *MTOR* mutations from this study are listed.

Supplementary Table 5 – All site-directed mutagenesis primers to generate mutant mTOR and Rheb1 are listed.