Supplementary material:

Supplementary table 1: Data collection and refinement statistics

Supplementary Fig. SI1: All tested FKBP-rapamycin complexes bind to FRB domain of mTOR with high affinity.

FRET binding assay with recombinant GST-FRB and eGFP-FKBPs: half-logarithmic plot of the rapamycin concentration against the observed FRET ratio (Em 520 nm/ Em 495 nm). 5 nM GST-FRB, 100 nM eGFP-FKBPs and 2.5 nM of a Terbium-labelled anti-GST antibody were used. Rapamycin was titrated from 0 to 5 µM (●FKBP12.6; ○FKBP12; ▼FKBP13; ΔFKBP25; ■FKBP51; □FKBP52).

Supplementary Fig. SI2: Six different FKBPs reduce mTOR activity with nanomolar IC_{50} values in a rapamycin-dependent manner.

FRET-based mTOR activity assay, containing purified truncated mTOR and eGFP-4E-BP1 as a substrate: half-logarithmic plot of a rapamycin titration (0 – 1000 nM) against observed FRET ratio (Em 520 nm / Em 495 nm) in presence of 100 nM FKBP protein (●FKBP12; ○FKBP12.6; ▲FKBP13; ΔFKBP25; ■FKBP51; □FKBP52).

Supplementary Fig. SI3: FKBP12 and FKBP51 FK1 mediate growth inhibition by rapamycin.

Growth curves demonstrate that FKBP12 and FKBP51 FK1 have a similar effect on yeast cells: In glucose medium (SD, which will repress expression of FKBPs) or galactose containing medium (SG, which will induce expression of FKBPs), the indicated yeast clones were grown at 30°C in the presence or the absence of rapamycin (5 µM final concentration). OD_{600} was measured every 180 minutes and plotted against time.

Supplementary Fig. SI4: In FKBP12-knockdown cells, rapamycin-induced Akt hyper-phosphorylation is reduced compared to wild type cells.

Cellular Akt phosphorylation was determined using a homogeneous time-resolved assay. SH-SY5Y neuroblastoma cells were starved for 24 h and stimulated with 10% FBS/100 nM insulin for 60 minutes in the presence of 0-3.5 nM rapamycin or 10 nM Torin-1. Mean values of three independent data points are shown.

Supplementary Fig. SI5: FKBP51 overexpression increases rapamycin-induced Akt phosphorylation in HeLa cells

HeLa cells were transfected for expression of FLAG-FKBP51 or empty plasmid. After 24 h, cells were starved for 24 h and then stimulated with serum and insulin for 60 minutes in presence of 0-25 nM rapamycin. Cells were lysed and subjected to immunoblotting analysis. (A) Western blot analysis and (B) densitometric analysis of phosphoS473 Akt signals (relative to load).

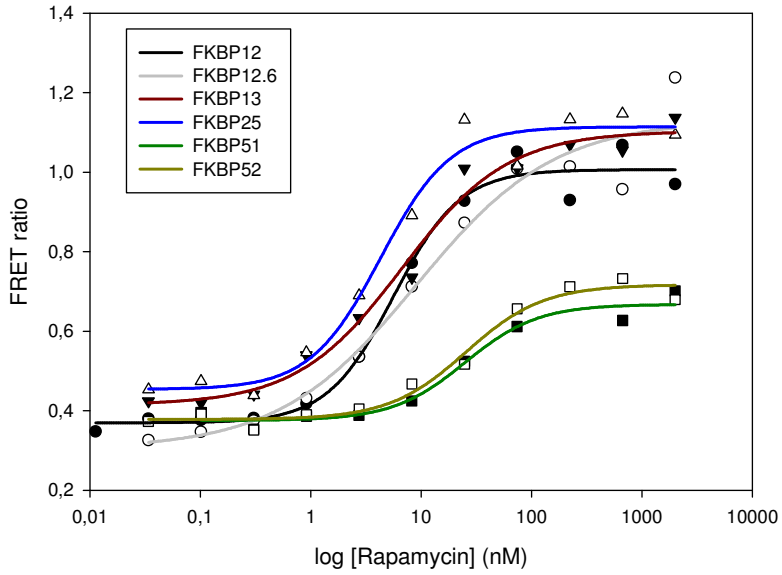
Supplementary images and tables:

Table S1:

	FKBP51 FK1-rapamycin-FRB	FKBP51 FK1-rapamycin-FRB (low pH)	FKBP52 FK1-rapamycin-FRB
Data collection			
Space group	$P2_12_12_1$	$P3_112$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	59.48, 59.52, 67.78;	103.68, 103.68, 106.54;	58.52, 62.99, 70.14;
α, β, γ (°)	90, 90, 90	90, 90, 120	90, 90, 90
Resolution (Å)	59.48 - 1.45 (1.53 - 1.45)*	89.80 - 2.3 (2.42 - 2.3)	70.19 - 1.8 (1.96 - 1.8)
R_{merge}	0.043 (0.377)	0.081 (0.519)	0.044 (0.350)
$I / \sigma I$	17.3 (3.3)	18.3 (4.1)	16.2 (3.0)
Completeness (%)	95.7 (93.8)	99.4 (98.8)	99.0 (98.9)
Redundancy	3.8 (3.5)	7.4 (7.5)	3.5 (3.6)
Refinement			
Resolution (Å)	19.05 - 1.45	20 - 2.3	19.76 - 1.8
No. reflections	39213	27545	23105
R_{work} / R_{free}	0.1769 / 0.2064	0.1871 / 0.2259	0.1883 / 0.2247
No. atoms			
Protein	1794	3517	1731
Ligand/ion	65	130 / 105	65 / 5
Water	223	89	151
B-factors			
Protein	24.56	45.31	40.78
Ligand/ion	15.74	34.78 / 86.55	24.44 / 54.82
Water	35.48	41.87	44.79
R.m.s. deviations			
Bond lengths (Å)	0.014	0.012	0.012
Bond angles (°)	1.546	1.450	1.287

For each structure a single crystal was used. *Values in parentheses are for highest-resolution shell.

SI1:


FKBP12
FKBP12.6
FKBP13
FKBP25
FKBP51
FKBP52
FRET ratio
log [Rapamycin] (nM)
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1,2
1,0
0,8
0,6
0,4
0,2
0,01
0,1
1
10
100
1000
10000

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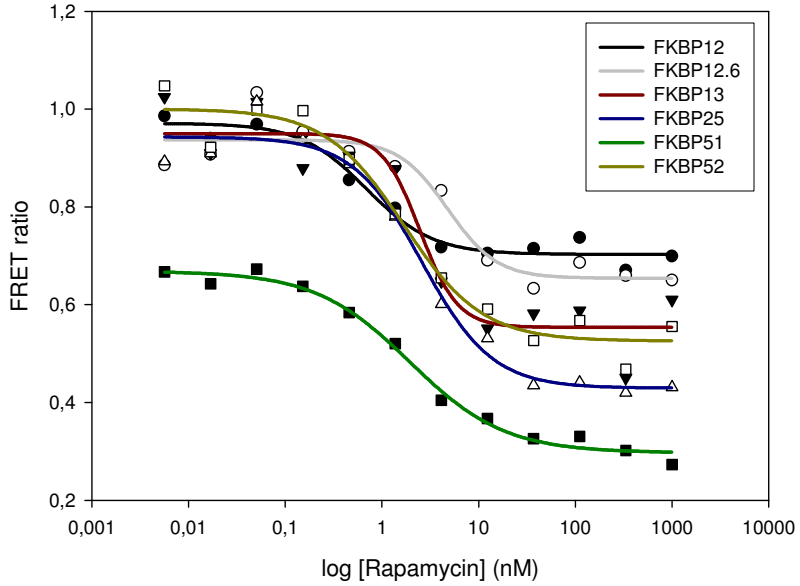
FKBP12
FKBP12.6
FKBP13
FKBP25
FKBP51
FKBP52
FRET ratio
log [Rapamycin] (nM)
1,2
1,0
0,8
0,6
0,4
0,2
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0,01
0,1
1
10
100
1000
10000

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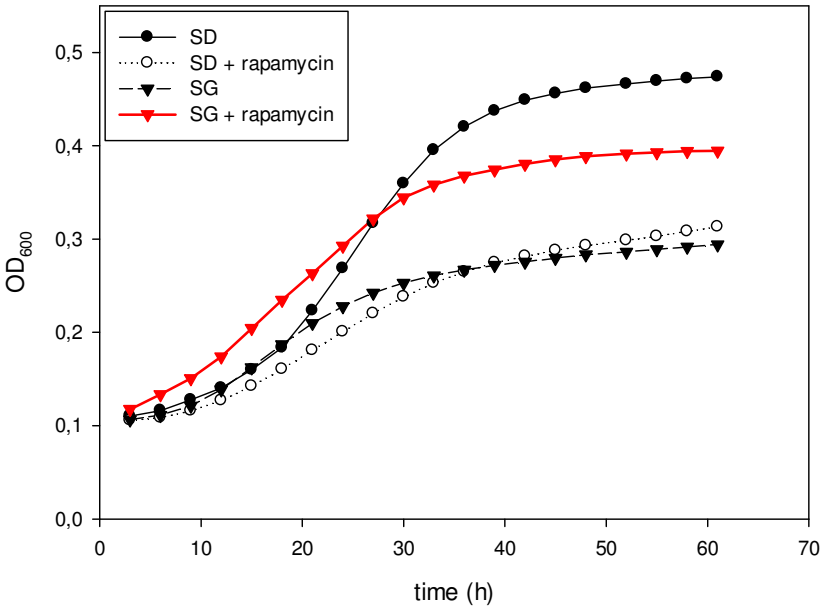
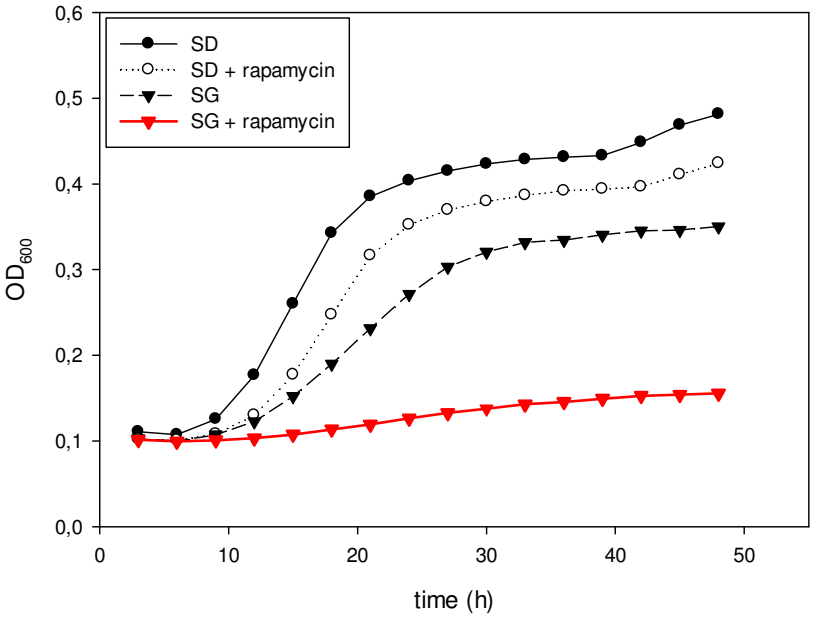
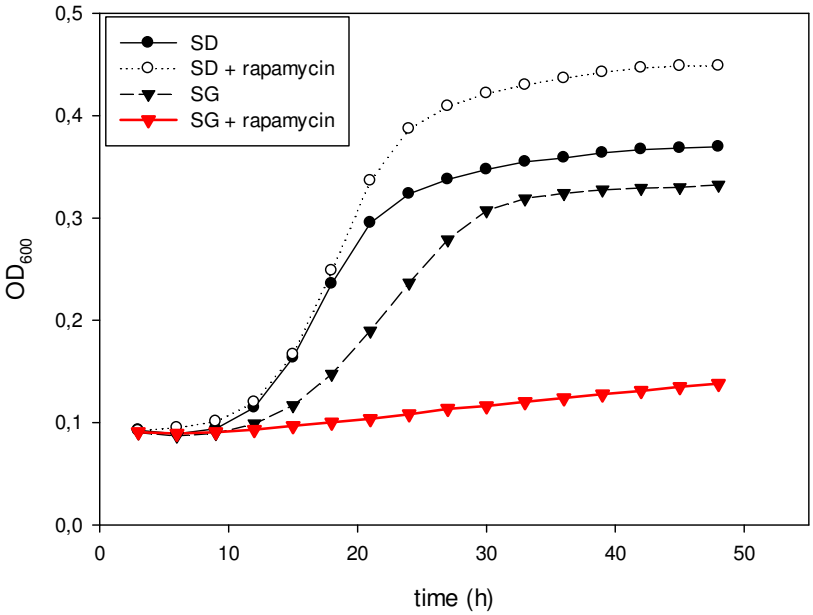


Deletion mutant Δ + empty vector

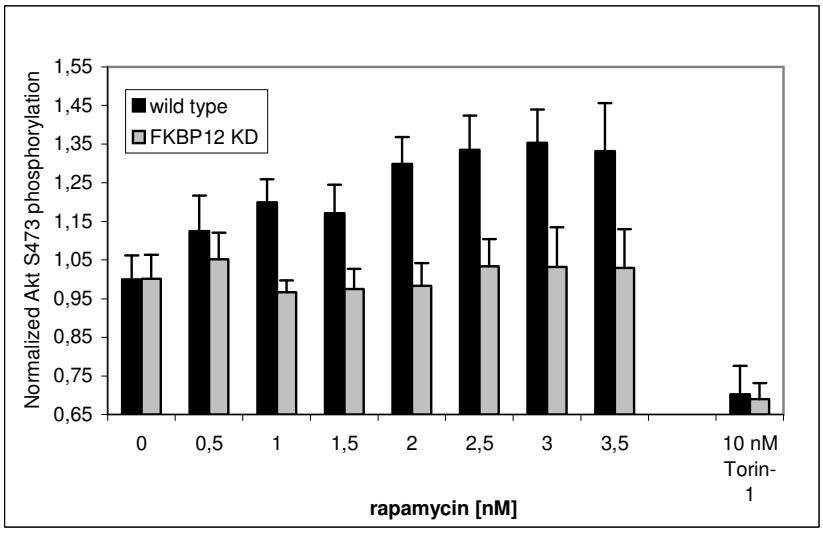


Δ + FKBP12



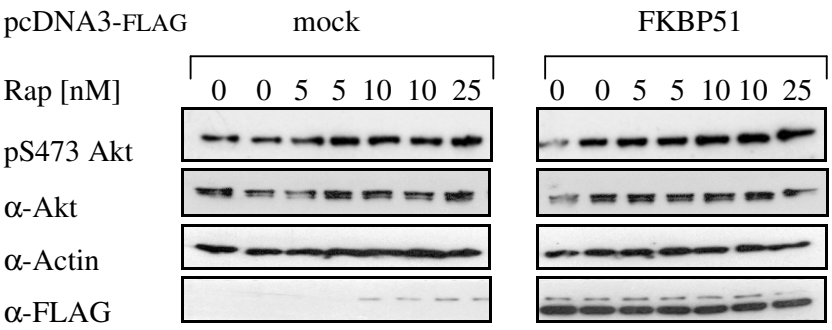
Δ + FKBP51 FK1

SI4:

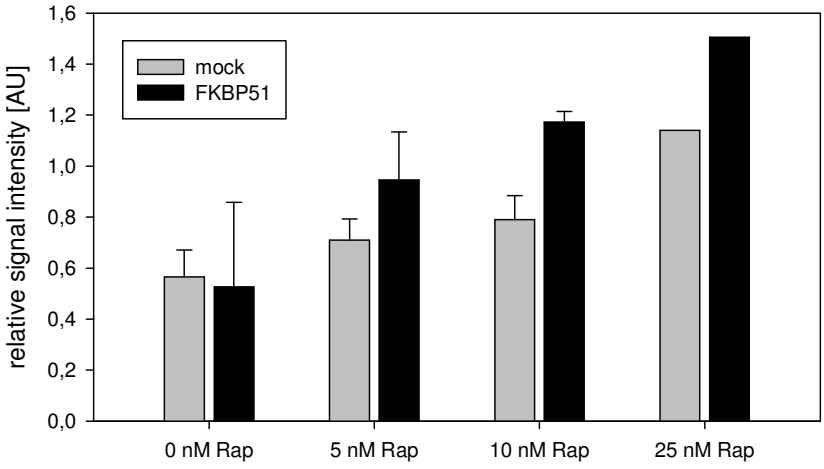


SI5:

A


pcDNA3-FLAG
mock
FKBP51
Rap [nM]
0 0 5 5 10 10 25
0 0 5 5 10 10 25
pS473 Akt
α-Akt
α-Actin
α-FLAG

B


relative signal intensity [AU]
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
mock
FKBP51
0 nM Rap
5 nM Rap
10 nM Rap
25 nM Rap