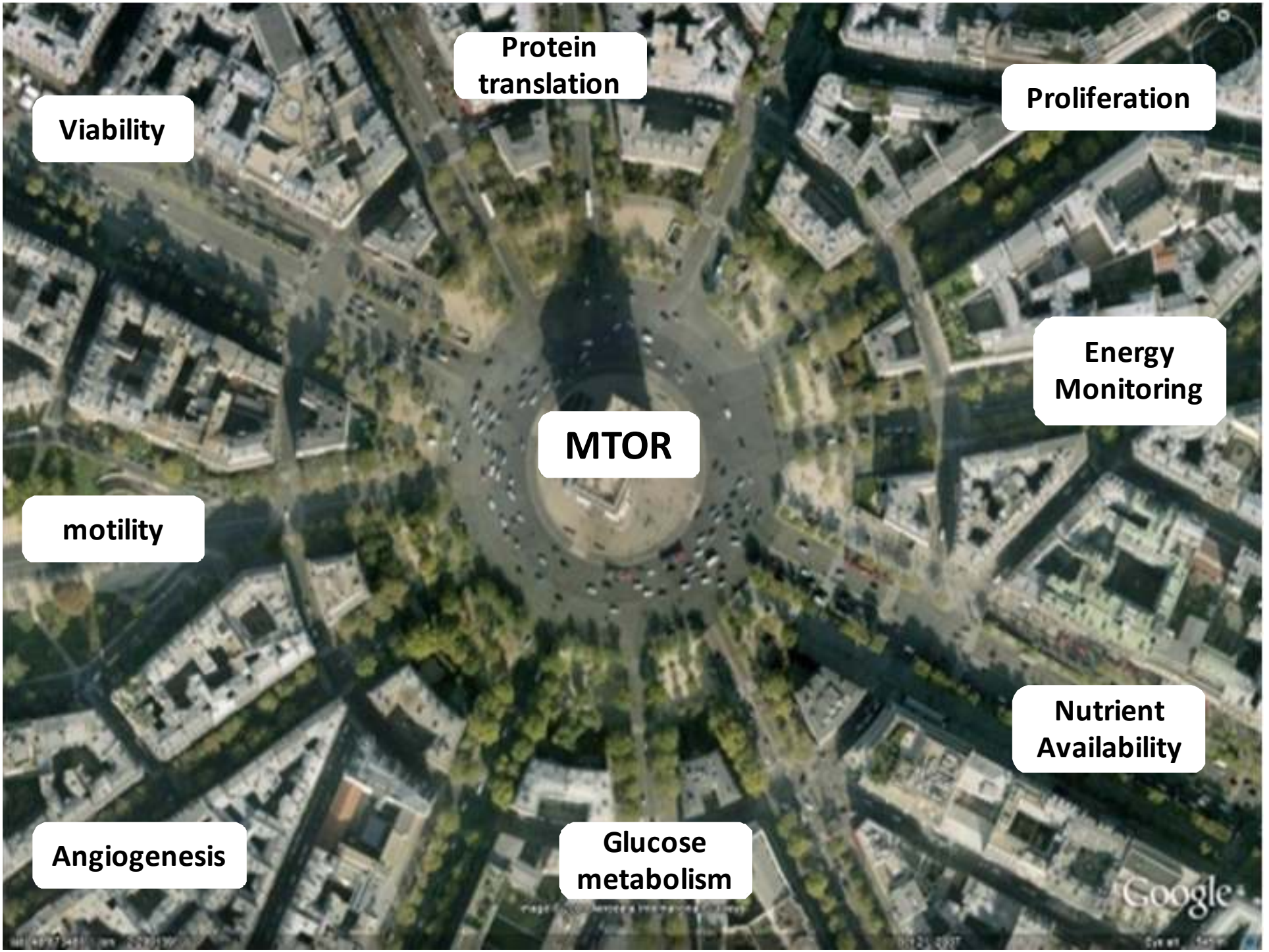
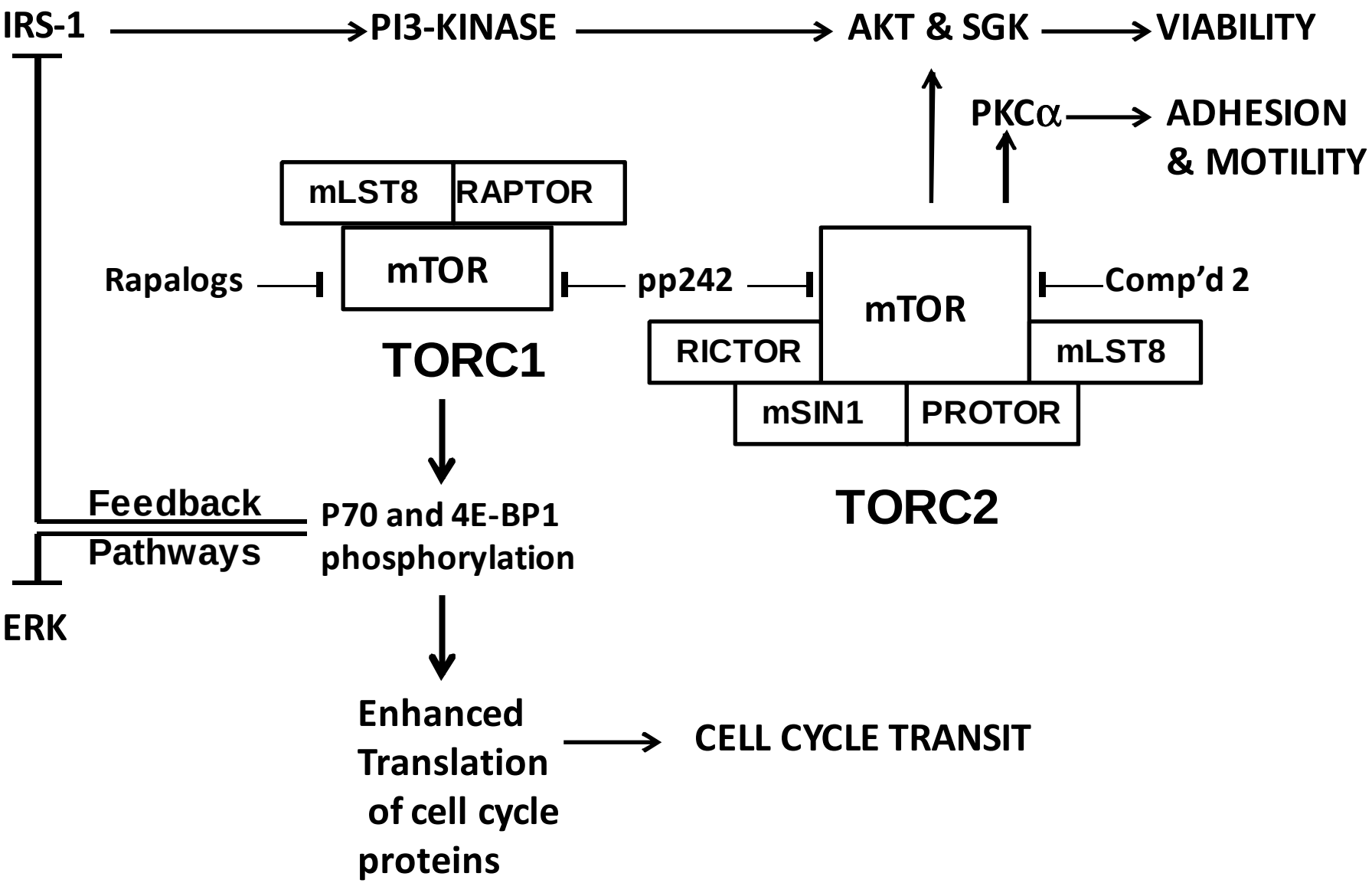
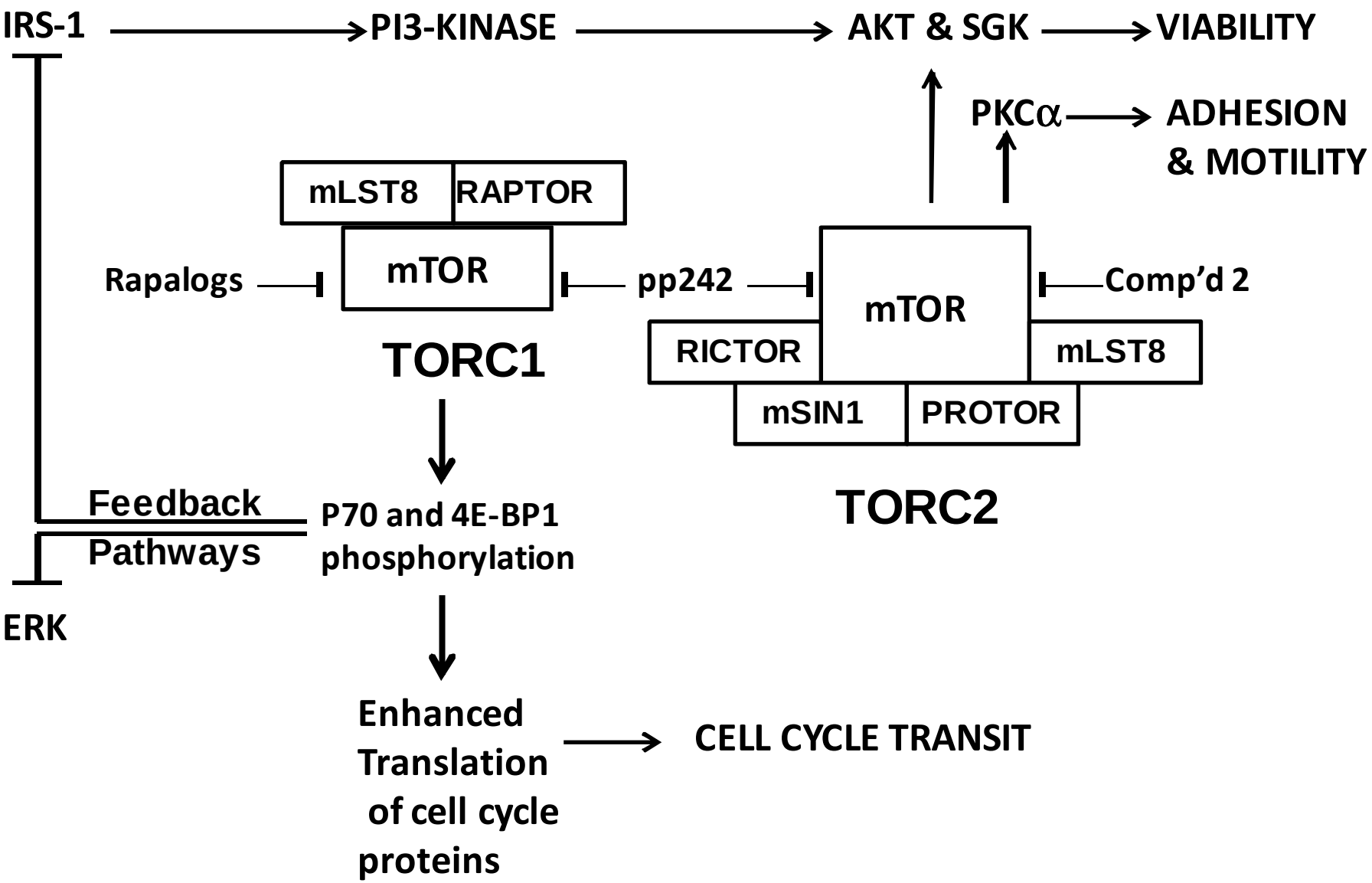
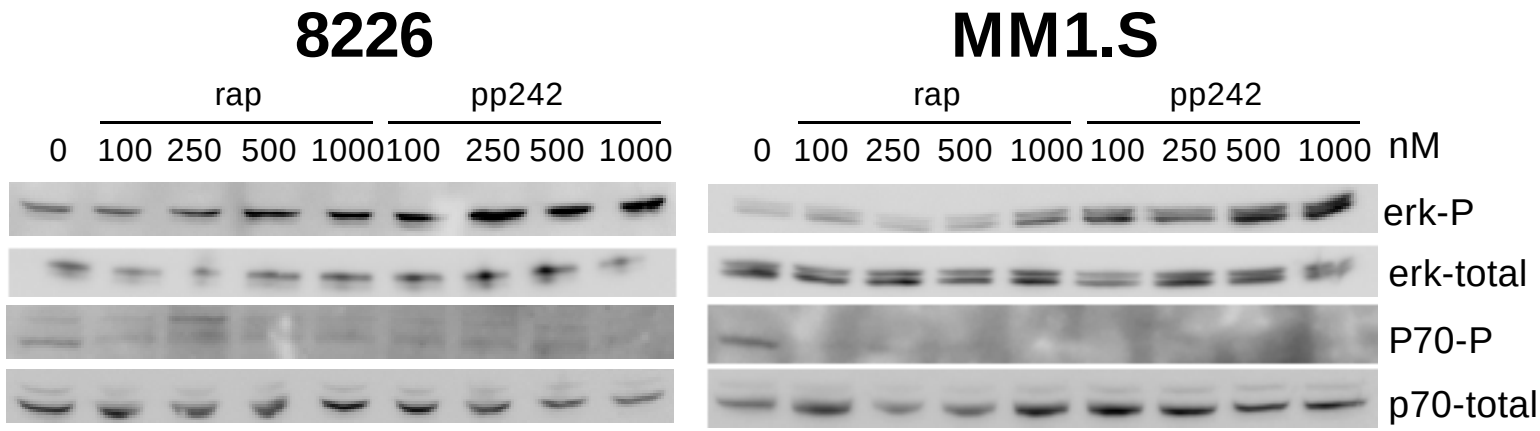

Protein translation
Proliferation
Viability
Energy Monitoring
MTOR
motility
Nutrient Availability
Angiogenesis
Glucose metabolism
Google

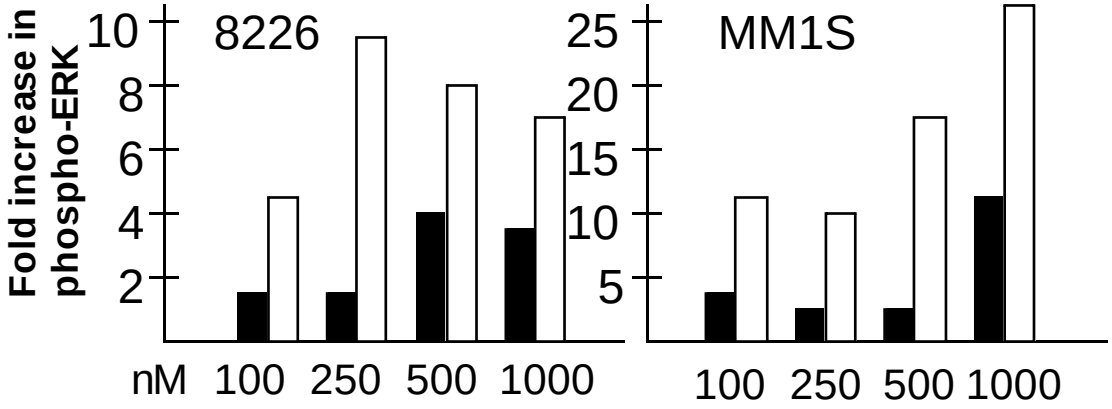

IRS-1
PI3-KINASE
AKT & SGK
VIABILITY
PKCα
ADHESION
& MOTILITY
mLST8
RAPTOR
mTOR
Rapalogs
pp242
mTOR
Comp'd 2
TORC1
RICTOR
mLST8
mSIN1
PROTOR
TORC2
Feedback
Pathways
P70 and 4E-BP1
phosphorylation
ERK
Enhanced
Translation
of cell cycle
proteins
CELL CYCLE TRANSIT

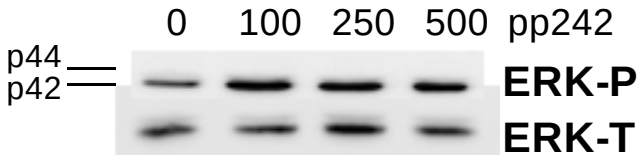
Active site mTOR inhibitors

- Inhibit mTOR at ATP-binding site
- Pp242, torin 1, INK 128, OSI are examples; In trials currently
- More active than rapamycin against MM cells in pre-clinical survival assays; Synergizes with bortezomib
- Inhibits TORC1- inhibits phosphorylation of p70 and 4E-BP1 and, thus, inhibits protein translation
- Inhibits TORC2- inhibits AKT phosphorylation and SGK1 activity
- Improved activity vs rapalogs thought due to more intense inhibition of 4E-BP1 phosphorylation and decreased protein translation
- However, rictor knock down (TORC2 paralysis) is deleterious to MM cells suggesting inhibition of TORC2 plays a role in enhanced efficacy

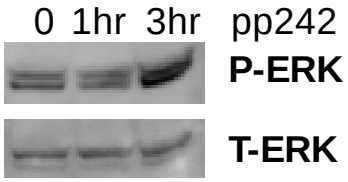

IRS-1
PI3-KINASE
AKT & SGK
VIABILITY
PKCα
ADHESION
& MOTILITY
mLST8
RAPTOR
mTOR
Rapalogs
pp242
mTOR
Comp'd 2
TORC1
RICTOR
mLST8
mSIN1
PROTOR
TORC2
Feedback
Pathways
P70 and 4E-BP1
phosphorylation
ERK
Enhanced
Translation
of cell cycle
proteins
CELL CYCLE TRANSIT







Patient #1



Patient #2

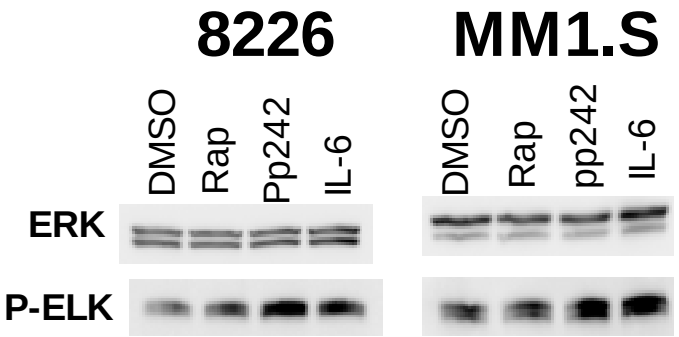
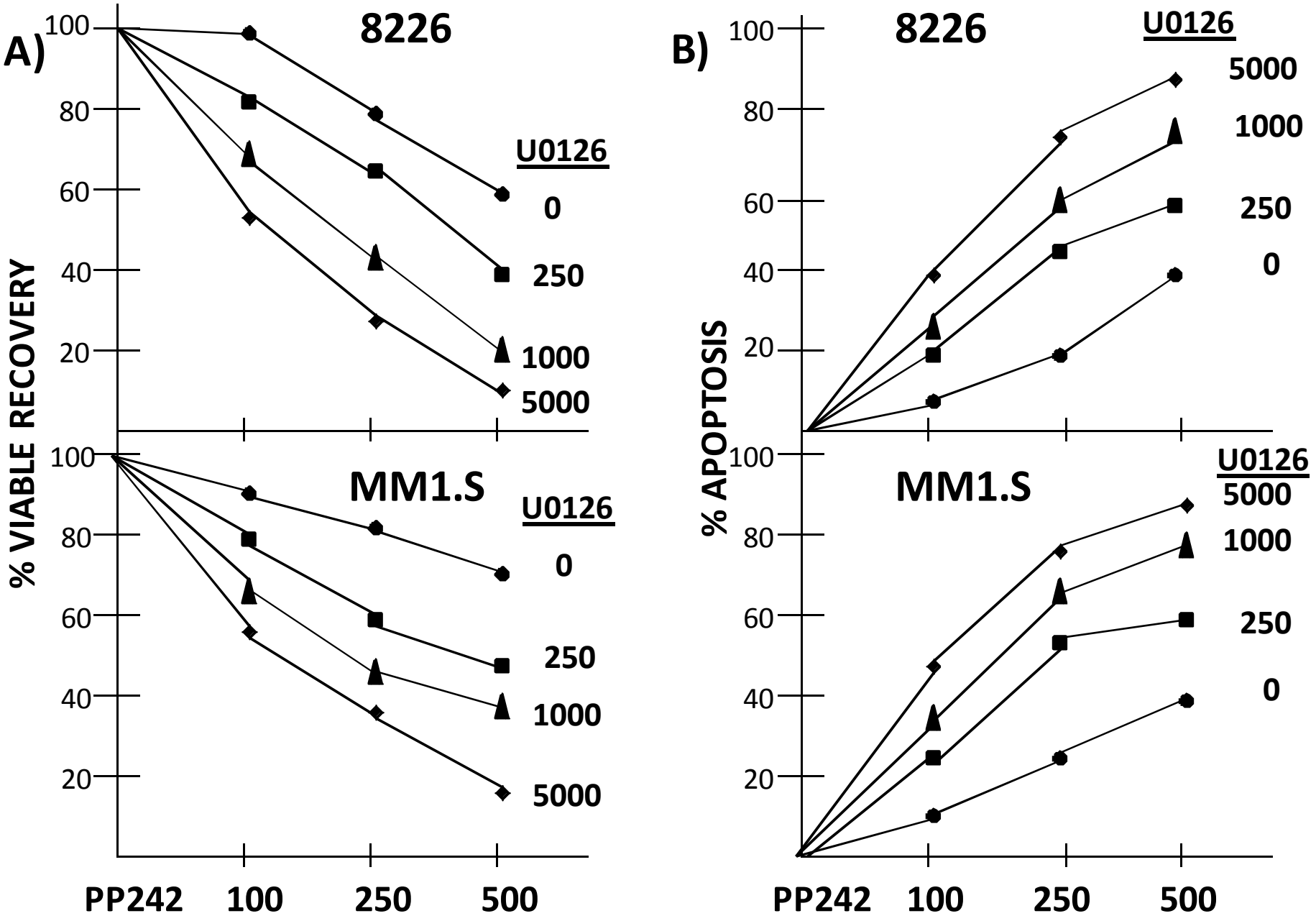
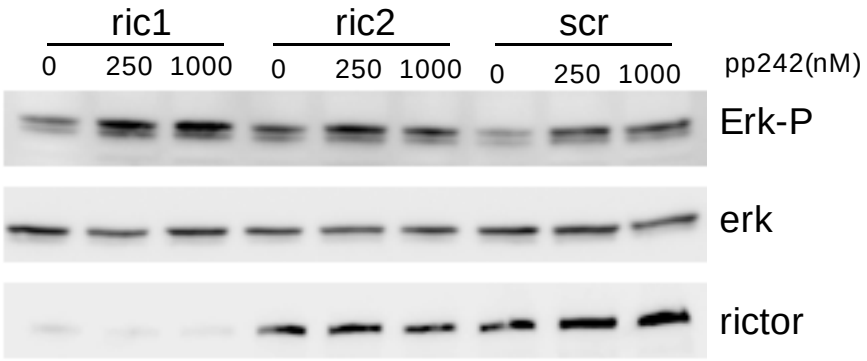


FIGURE 6

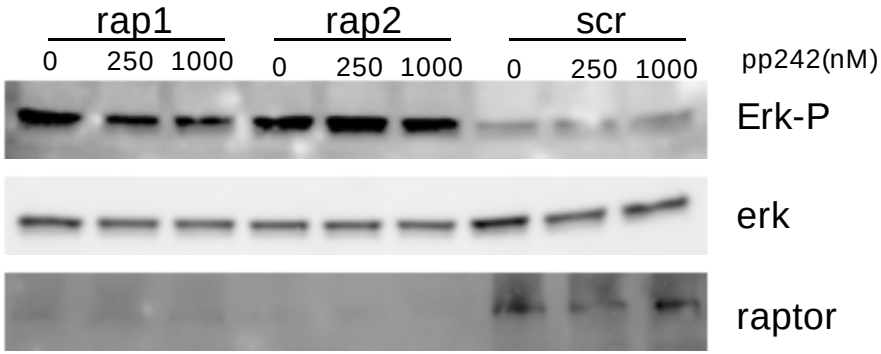

A)
8226
MM1.S
% VIABLE RECOVERY
100
80
60
40
20
U0126
0
250
1000
5000
PP242
100
250
500
B)
8226
MM1.S
% APOPTOSIS
U0126
5000
1000
250
0

8226

A)


ric1
ric2
scr
0 250 1000 0 250 1000 0 250 1000
pp242(nM)
Erk-P
erk
rictor

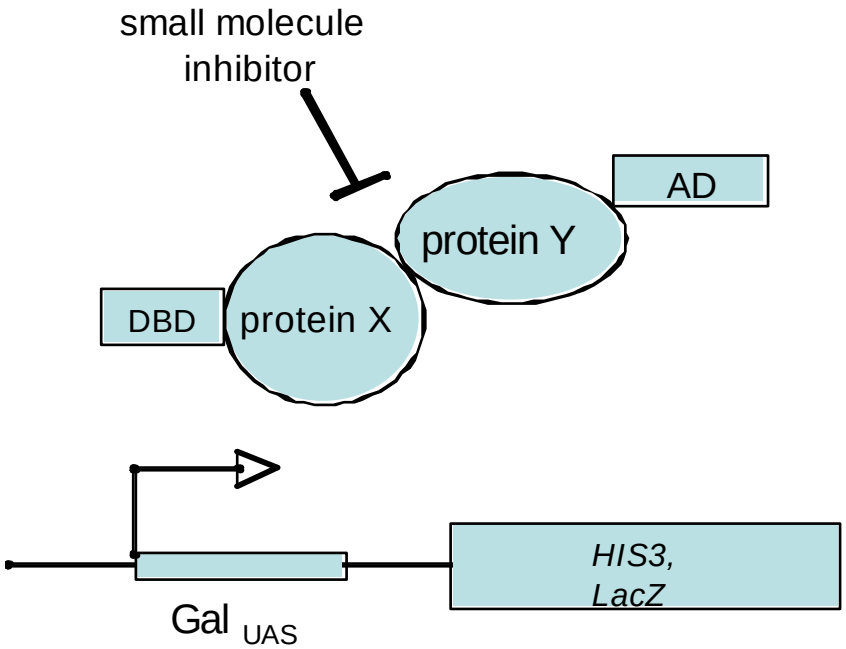
B)


rap1
rap2
scr
0 250 1000 0 250 1000 0 250 1000
pp242(nM)
Erk-P
erk
raptor

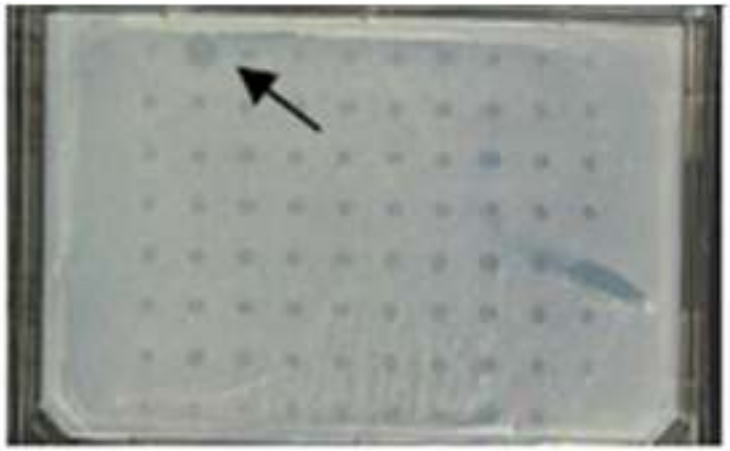
HIGH THROUGHPUT SCREEN FOR INHIBITOR OF RICTOR-mTOR INTERACTION

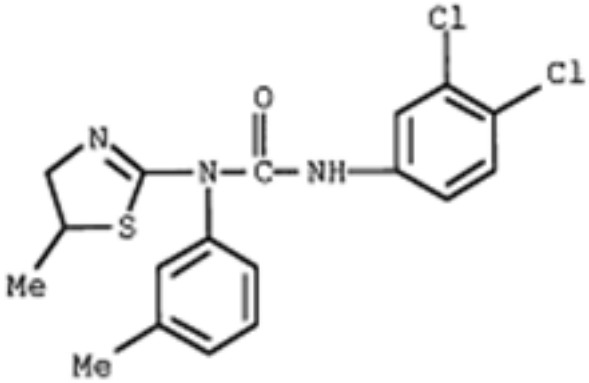
1) MAKE YEAST HYPERPERMEABLE TO SMALL INHIBITORS

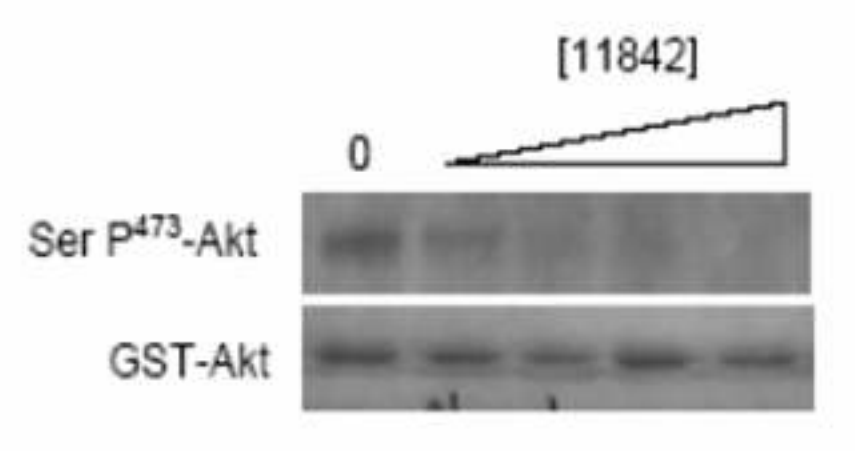
2) CLONE mTOR AND RICTOR INTO YEAST VECTORS FOR TWO-HYBRID ASSAY

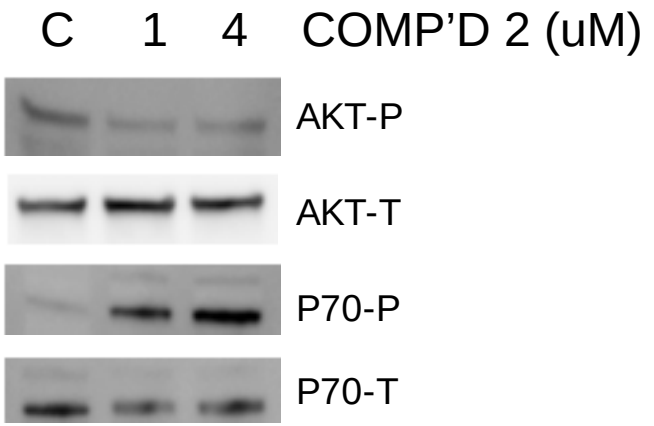


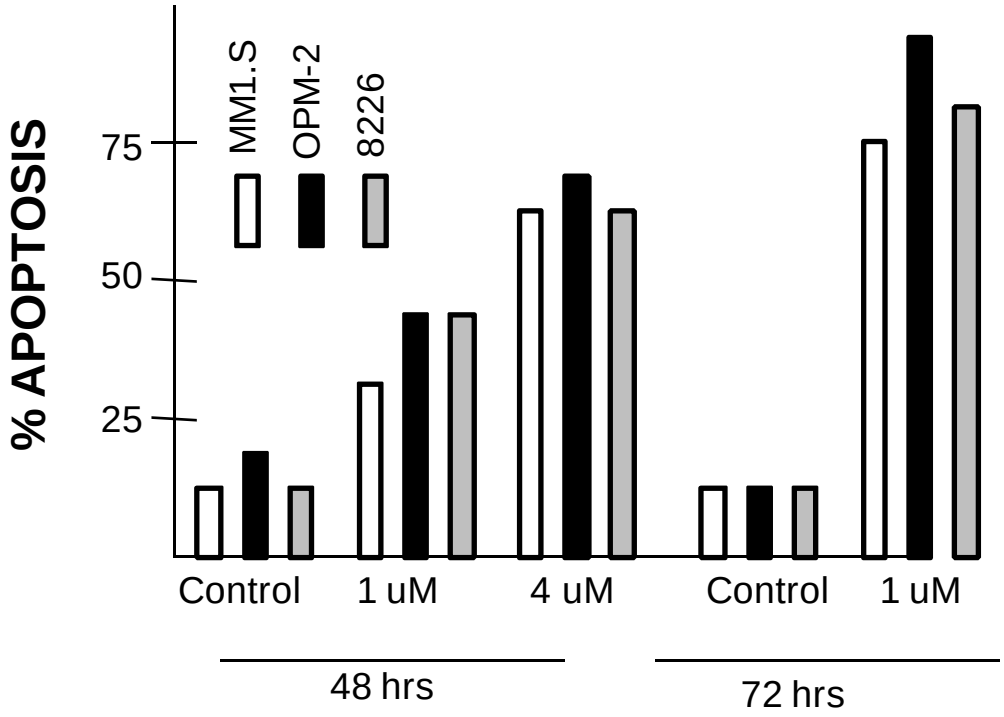
COMPONENT 1	COMPONENT 2	CLONE 1	CLONE 2
DBD			
	AD		
	RICTOR AD		
DBD mTOR			
DBD mTOR	RICTOR AD		

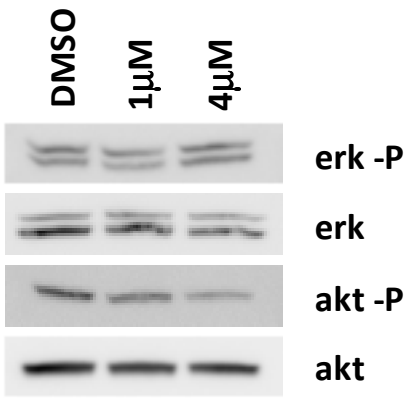





[11842]
0
Ser P473-Akt
GST-Akt


C 1 4 COMP'D 2 (uM)
AKT-P
AKT-T
P70-P
P70-T


% APOPTOSIS
75
50
25
MM1.S
OPM-2
8226
Control
1 uM
4 uM
Control
1 uM
48 hrs
72 hrs

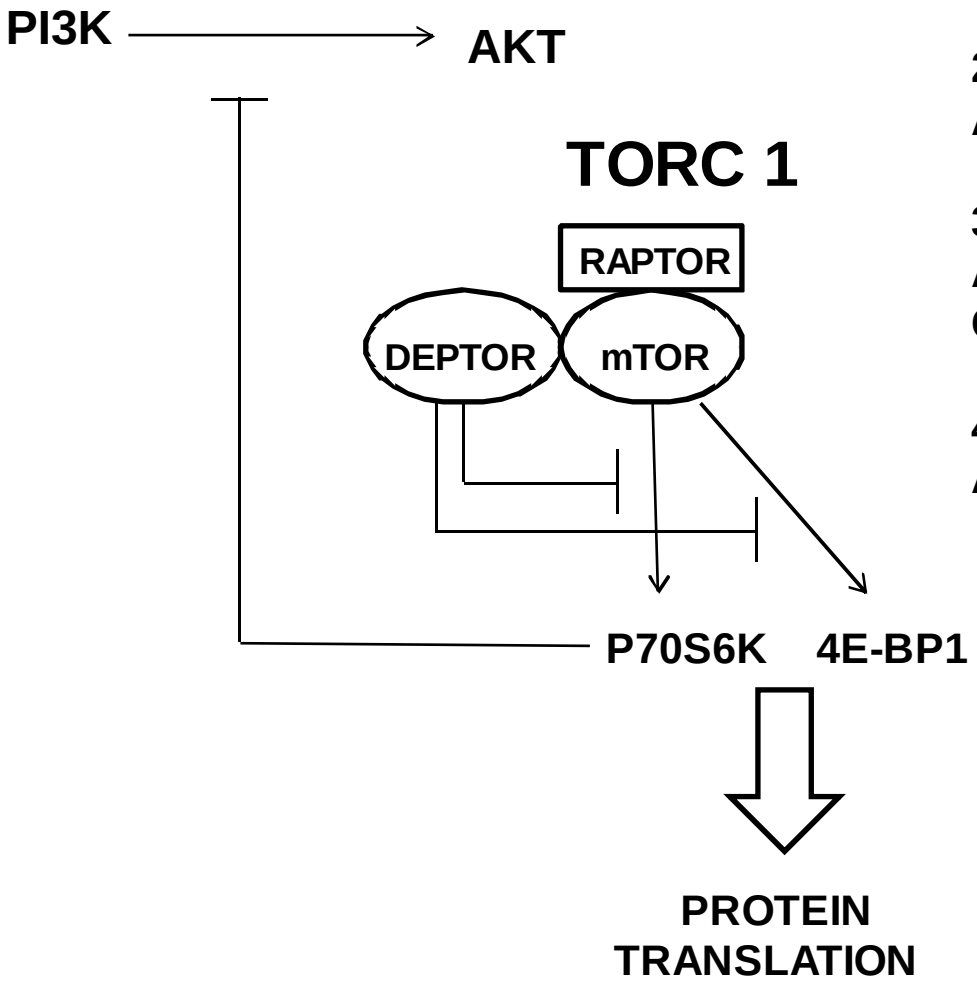

DMSO
1μM
4μM
erk -P
erk
akt -P
akt

CONCLUSIONS

- **Although active site TOR kinase inhibitors are better than rapalogs against MM cells, they induce ERK activation**
- **ERK activation functions as a mechanism of resistance**
- **Identification of a selective TORC2 inhibitor, by a high throughput yeast-two-hybrid assay against mTOR-RICTOR binding, demonstrates the induction of MM apoptosis without activation of ERK**

DEPTOR

- A 48 kDa protein that binds to mTOR within both TORC1 and TORC2 complexes
- Negatively regulates both TORC1 and TORC2 activity
- Not significantly expressed in any malignancies other than myeloma
- Over-expressed in 28% of myeloma specimens
- Over-expression specifically found in cases with Ig translocations
- Is a MAF target, explaining especially high expression in MAF-translocated myeloma
- Its inhibition of TORC1 results in marked feedback activation of PI3-K/AKT
- Knockdown results in prevention of MM cell growth and apoptosis



Ramifications of high DEPTOR expression

1. **INHIBITED PROTEIN TRANSLATION INDUCING PROTECTION AGAINST ER STRESS**

2. **CAUSE OF HIGH BASAL AKT ACTIVATION**

3. **POSSIBLE MECHANISM OF ANTI-APOPTOTIC SIGNALING IN MYELOMA CELLS**

4. **POTENTIAL INDUCER OF AKT ADDICTION IN MYELOMA CELLS**

WHO DID THE WORK

- Joseph Gera
- Bao Hoang
- Angie Benavides
- Yijiang Shi
- Yonghui Yang

- SUPPORTED BY
- 1) NIH
- 2) DOD
- 3) VA
- 4) MMRF