Figure S1. Fluctuation of the levels of Mxi1 during cell cycle progression. HeLa cells were synchronized in S phase with hydroxyurea (HU) for 24 h and then were released. Cells were harvested at indicated time points and subjected to immunoblot analyses using indicated antibodies (n=3).

Figure S2. Knockdown of S6K1 but not RSK stabilizes endogenous Mxi1. HeLa cells were transfected with indicated siRNAs for 48 h and then analyzed by Western blotting as indicated (n=3). The ratio shows relative Mxi1 protein expression normalized for GAPDH (control, set at 1).

Figure S3. The mRNA levels of Mxi1 were analyzed by Real time-PCR in A549 and H1299 cells stably expressing wild-type or mutant Mxi1. n.s. indicates no statistically significant difference ($P > 0.05$). (n=3).

Figure S4. Immunoblots showing levels of S6K1, p-S6K1, β-Trcp1, Myc and Max in lysates prepared from five different lung cancer cell lines. Cells were harvested and lysates were blotted with indicated antibodies (n=3).

Figure S5. A. Alignment of S6K1 and β-Trcp phosphodegron motifs in four Mad family members (Mad1, Mxi1, Mad3 and Mad4) from human. **B.** HEK293T cells were transfected with the indicated constructs for 24h. Cells were collected and then analyzed with indicated antibodies (n=3). The ratio shows relative Mad family protein expression

normalized for GAPDH (control, set at 1)

Figure S1.

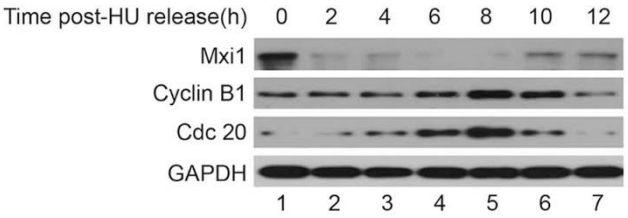

Time post-HU release(h) 0 2 4 6 8 10 12
Mxi1
Cyclin B1
Cdc 20
GAPDH
1 2 3 4 5 6 7

Figure S2.

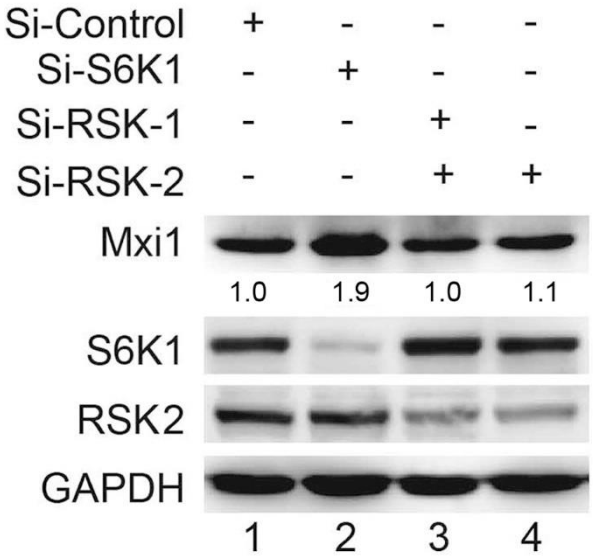

Si-Control + - - -
Si-S6K1 - + - -
Si-RSK-1 - - + -
Si-RSK-2 - - + +
Mxi1
1.0 1.9 1.0 1.1
S6K1
RSK2
GAPDH
1 2 3 4

Figure S3.

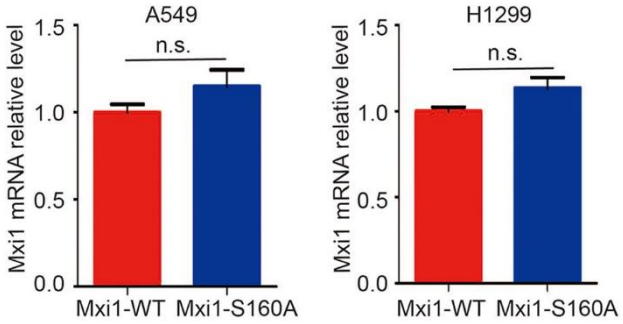

A549
n.s.
Mxi1 mRNA relative level
1.5
1.0
0.5
0.0
Mxi1-WT
Mxi1-S160A
H1299
n.s.
Mxi1 mRNA relative level
1.5
1.0
0.5
0.0
Mxi1-WT
Mxi1-S160A

Figure S4.

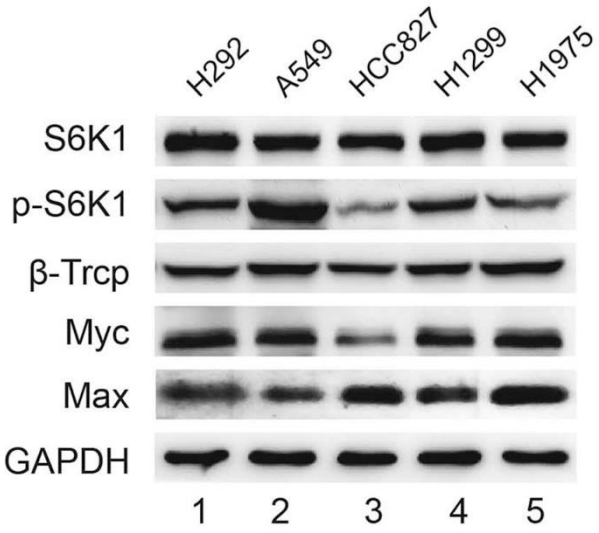
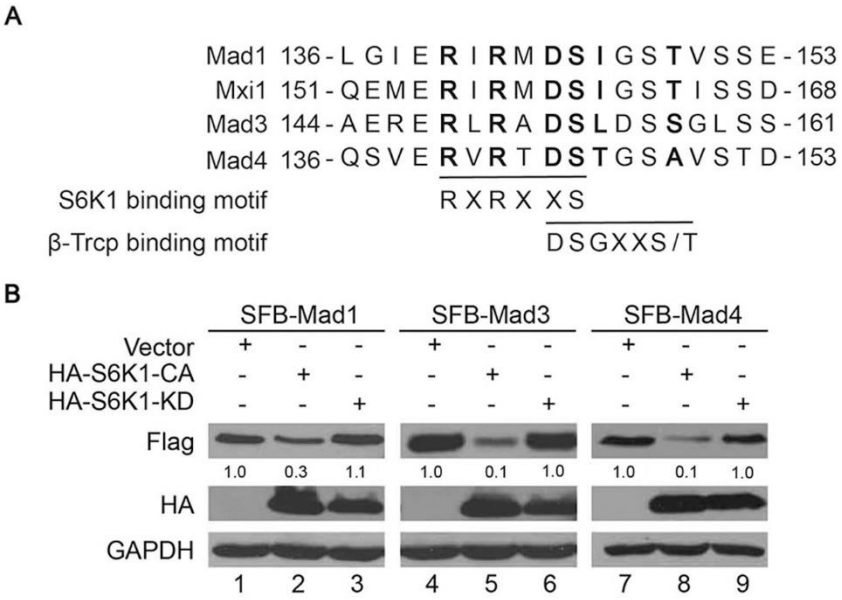

H292
A549
HCC827
H1299
H1975
S6K1
p-S6K1
β-Trcp
Myc
Max
GAPDH
1
2
3
4
5

Figure S5.


A
Mad1 136 - L G I E R I R M D S I G S T V S S E - 153
Mxi1 151 - Q E M E R I R M D S I G S T I S S D - 168
Mad3 144 - A E R E R L R A D S L D S S G L S S - 161
Mad4 136 - Q S V E R V R T D S T G S A V S T D - 153
S6K1 binding motif
R X R X X S
β-Trcp binding motif
D S G X X S / T
B
SFB-Mad1
SFB-Mad3
SFB-Mad4
Vector
HA-S6K1-CA
HA-S6K1-KD
Flag
1.0 0.3 1.1 1.0 0.1 1.0 1.0 0.1 1.0
HA
GAPDH
1 2 3 4 5 6 7 8 9