SUPPLEMENTAL MATERIALS AND METHODS

GST pull-down assay. GST and GST-FIH-1 recombinant proteins were prepared as previously described (1). Wild-type (WT) and TS/AA HF-Mint3 proteins were collected from lysates of HT1080 cells expressing WT or TS/AA HF-Mint3 by immunoprecipitation using anti-FLAG M2 antibody-conjugated beads (Sigma) followed by elution with FLAG peptides. Glutathione-Sepharose 4B (GE Healthcare)-conjugated GST fusion proteins (≈10 μg) were preincubated with 0.5 mg ml bovine serum albumin in lysis buffer for 30 min at 4 °C. Thereafter, WT or TS/AA HF-Mint3 was re-suspended in lysis buffer containing 0.5 mg/ml bovine serum albumin and added to the beads. After mixing by rotation for 2 h, the beads were washed four times with lysis buffer. The proteins were eluted with Laemmli sample buffer and analyzed by SDS-PAGE, followed by immunoblot analysis using anti-FLAG antibody (SIGMA) or anti-GST antibody (GeneTex, Irvine, CA).

HIF-2α reporter assay. pcDNA3.1 Gal4BD-HIF2α CAD (encoding amino acids 771-870) plasmid was prepared using a PCR based method. The reporter assay was performed as described in the main text.

REFERENCES

1. **Sakamoto, T., and M. Seiki.** 2009. Mint3 enhances the activity of hypoxia-inducible factor-1 (HIF-1) in macrophages by suppressing the activity of factor inhibiting HIF-1. J Biol Chem **284:**30350-30359.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. mTOR and MT1-MMP cooperatively boost HIF-1α activity in HEK293 cells
Reporter assays were performed in the presence or absence of rapamycin in HEK293 cells. The results of the reporter assays are shown. The data were analysed using the Student's t-test. **p < 0.01.

Figure S2. Posttranslational modification of Mint3
Areas enclosed by dashed lines in Fig. 3A, 3B, and 3D were magnified (left) and analysed by densitometry (right).

Figure S3. Posttranslational modification of HF-Mint3
Areas enclosed by dashed lines in Fig. 4A, 4D, 4F, and 4G were magnified (left) and analysed by densitometry (right).

Figure S4. mTOR regulates the translational modification of Mint3 in HEK293 cells
Rapamycin treatment increased the isoelectric point of the Mint3 protein. Relative intensity of each Mint3 signal is shown above the spot.

Figure S5. Phosphorylation of Mint3 by mTOR does not directly affect the binding affinity of Mint3 protein to FIH-1
A pull-down assay was performed using GST or GST-FIH-1 beads.

Figure S6. The MT1-MMP/Mint3 axis moderately promotes HIF-2α activity
(A, B) Reporter assays were performed in the presence or absence of rapamycin in HEK293 cells (A) or in control (shLacZ), Mint3 knocked-down, and MT1-MMP knocked-down HT1080 cells

(B). The results of the reporter assay**s** are shown. The data were analysed using the Student's t-test. *$p < 0.05$, **$p < 0.01$.

Figure S1

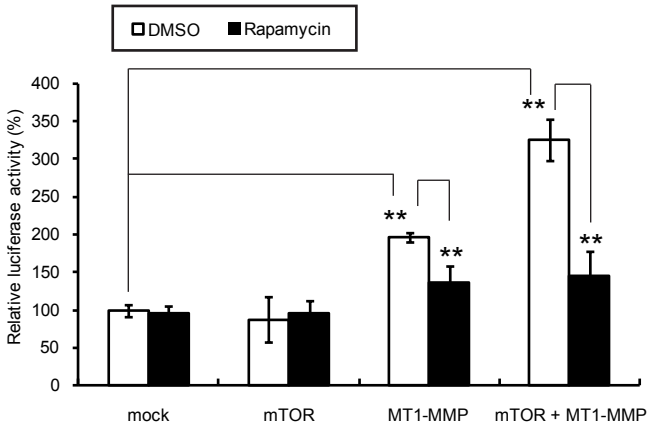
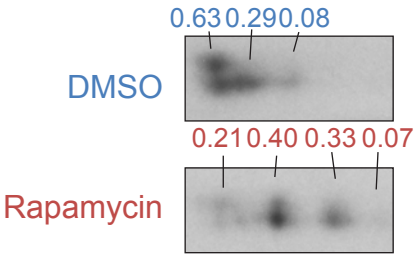

DMSO
Rapamycin
Relative luciferase activity (%)
400
350
300
250
200
150
100
50
0
**
**
**
**
mock
mTOR
MT1-MMP
mTOR + MT1-MMP

Figure S2

Fig. 3A



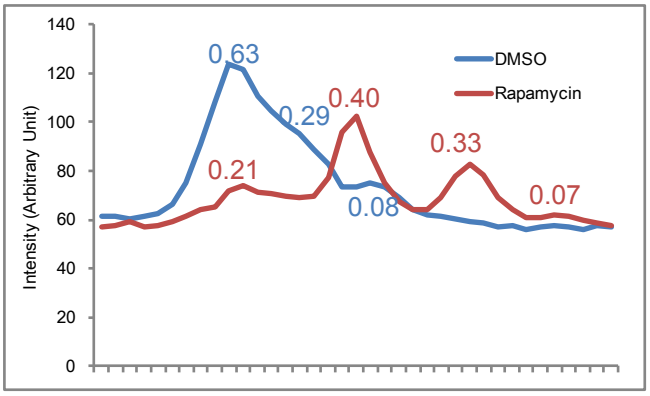
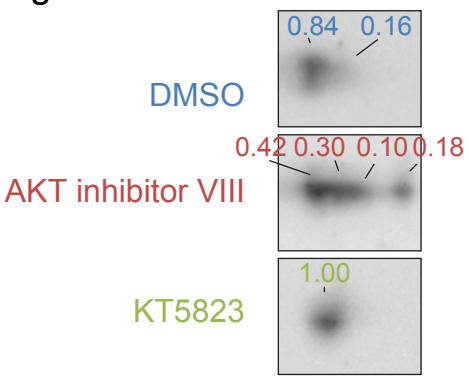


Fig. 3B



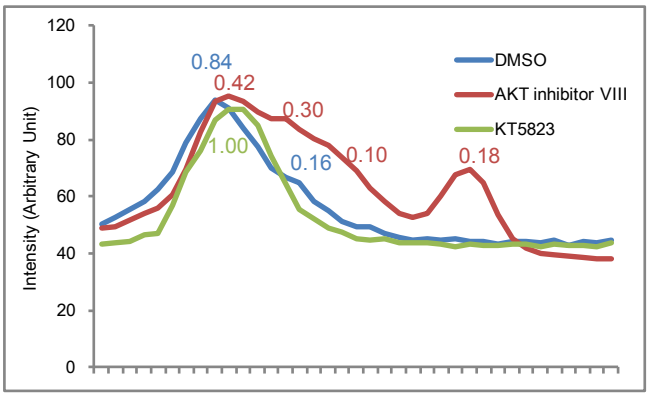
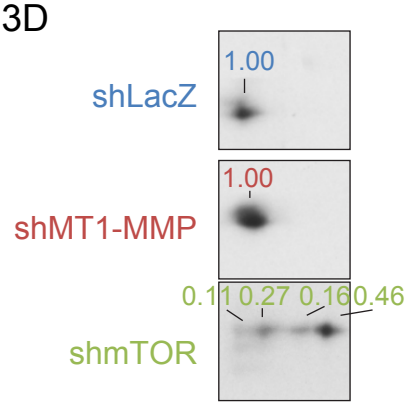


Fig. 3D



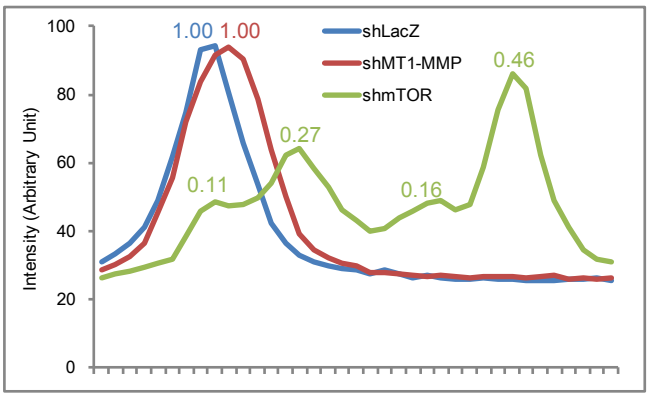
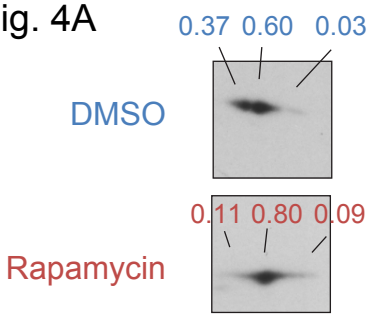


Figure S3

Fig. 4A



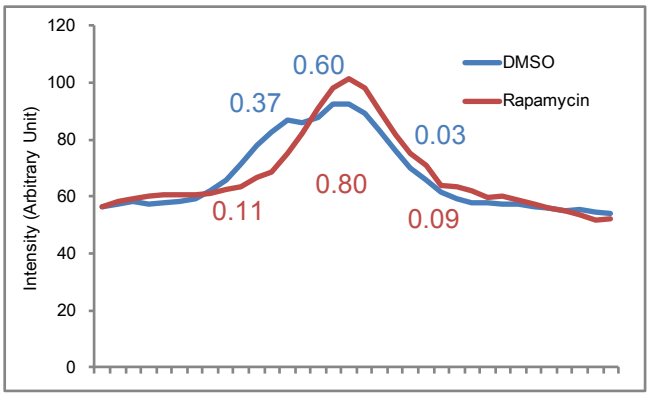
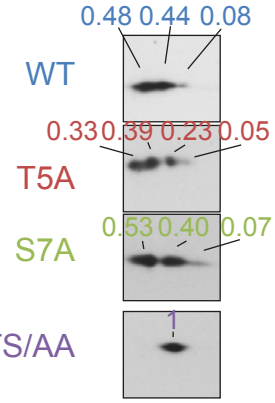


Fig. 4D



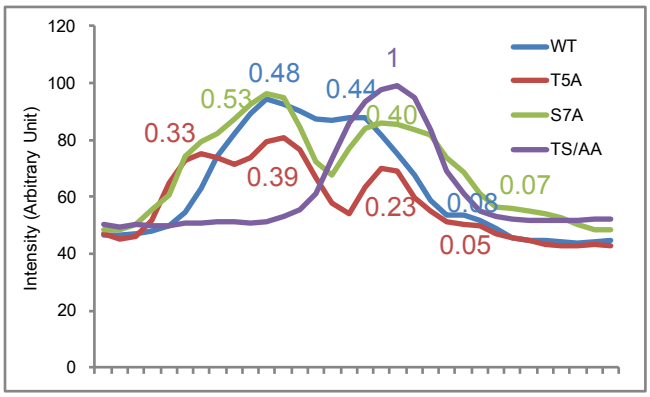
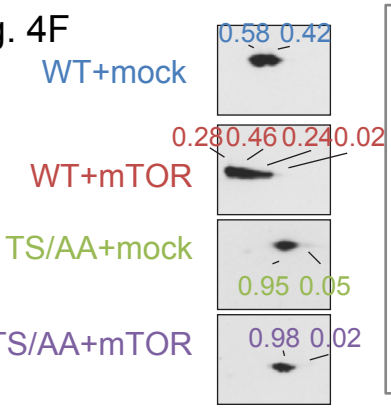


Fig. 4F



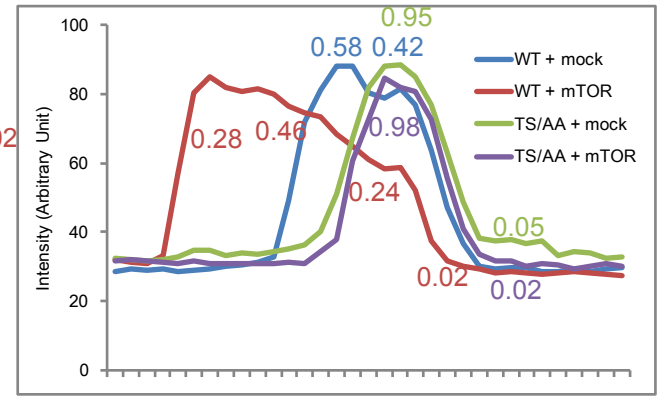
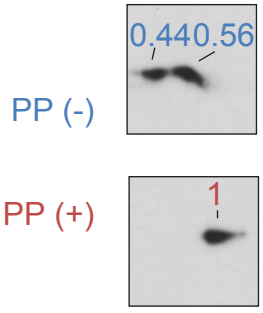


Fig. 4G



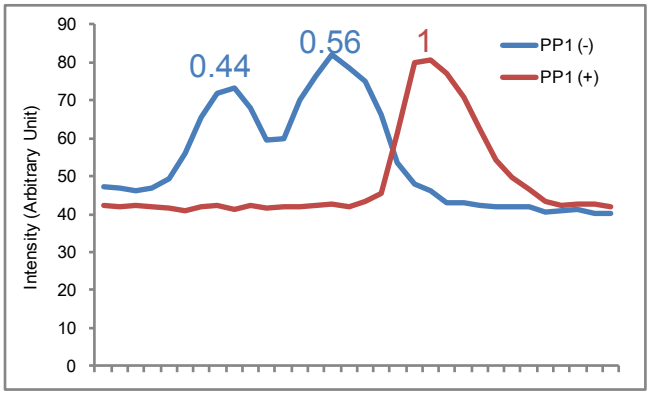


Figure S4

HEK293:

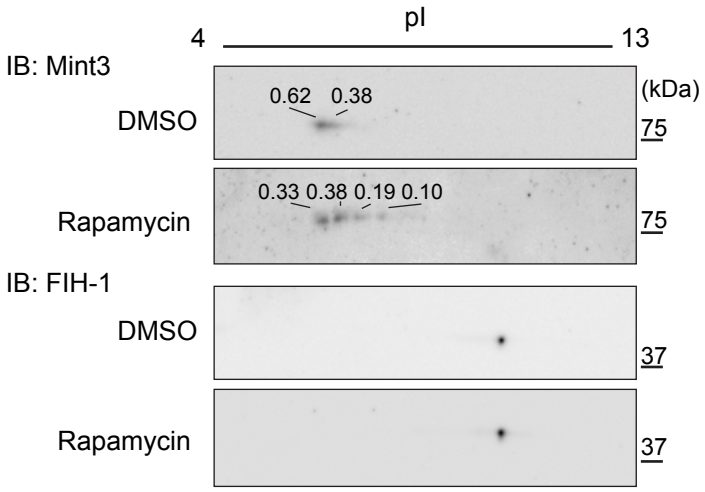


Figure S5

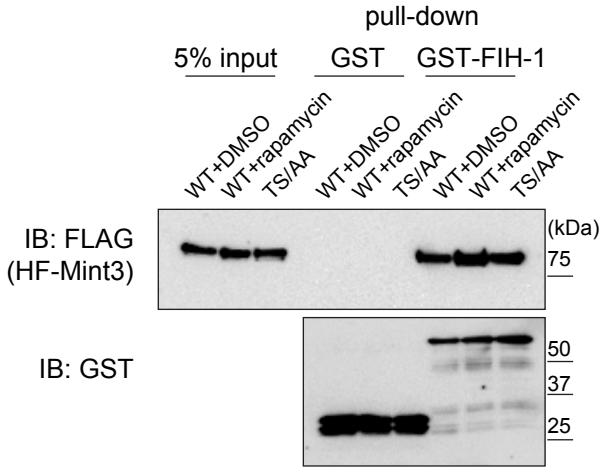
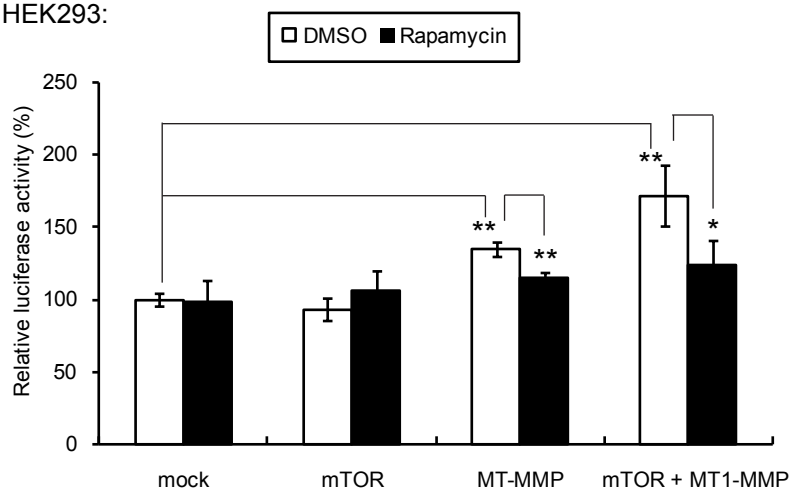

pull-down
5% input
GST
GST-FIH-1
WT+DMSO
WT+rapamycin
TS/AA
WT+DMSO
WT+rapamycin
TS/AA
WT+DMSO
WT+rapamycin
TS/AA
IB: FLAG
(HF-Mint3)
(kDa)
75
IB: GST
50
37
25

Figure S6

HIF-2α activity

A

HEK293:



B

HT1080:

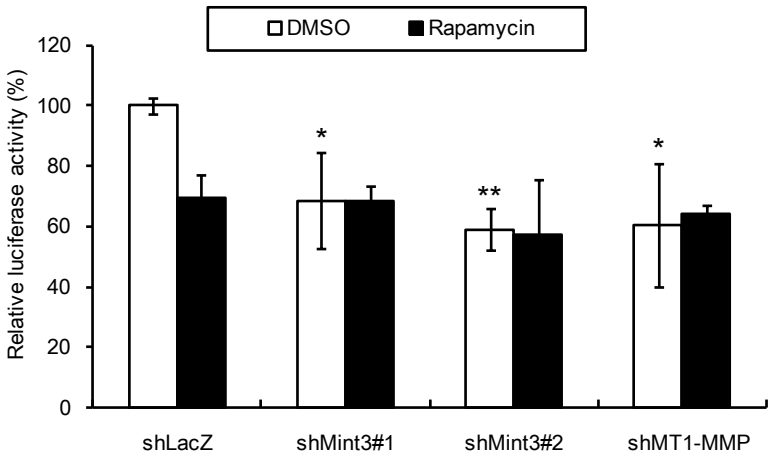


Table S1

MT1-MMP		5’-gcagccucucacuacucuuuccgaagaaagaguagugagaggcugc-3’
Mint3	#1	gaugauggcgguggugacgggacgaaucccgucaccaccgccauca
	#2	gcgguucuugguccuguaugacgaaucauacaggaccaagaaccgc
mTOR	#1	gcaaagaucucaugggcuucgcgaacgaagcccaugagaucuuugc
	#2	gcauccagcaggauaucaaggcgaaccuugauauccugcuggaugc
Raptor	#1	ggaaaccaagagagaggaagacgaaucuuccucucucuugguuucc
	#2	gcgaauugagacggacuuugacgaaucaaaguccgucucaauucgc
Rictor	#1	gcuauaacugcugguauuagacgaaucuaauaccagcaguuauagc
	#2	gccuuuauccauuugugaagucgaaacuucacaaauggauaaaggc
S6K1	#1	gcuugugauacucuugauacucgaaaguaucaagaguaucacaagc
	#2	gcacacaccuaugcaauauggcgaaccauauugcauaggugugugc
4E-BP1	#1	ggaaguggacaagaacgaacccgaagguucguucuuguccacuucc
	#2	guucguucuuguccacuuccugcgaacaggaaguggacaagaacgaa
Lipin1	#1	gcuaggaguuggguugcauuugcgaacaaaugcacccaacuccuagc
	#2	gcucaaggcuggaauauuuaucgaaauaaaauauuccagccuugagc
CLIP1	#1	gcuaaagccaaguggggcuuaacgaauuaagcccacuuggcuuuagc
	#2	ggaguuagaugagccacuuggcgaaccaaguggcucaucuaacucc
STAT3	#1	gcuacauacuccuggcauugccgaagcaaugccaggaguauguagc
	#2	gcacuuguaauggcgucuucacgaaugaagacgccauuacaagugc

Table S2

β-actin	Sense (S)	5’-GGGACGACATGGAGAAAATC-3’
	Antisense (A)	5’-GGGTGTTGAAGGTCTCAAAC-3’
GLUT1	S	GGGCATGTGCTTCCAGTATGT
	A	ACCAGGAGCACAGTGAAGAT
HK2	S	GTCCACTCCAGATGGGACAG
	A	GGAGCCCATTGTCCGTTACT
PGK1	S	GCATACCTGCTGGCTGGATG
	A	CCCACAGGACCATTCCACAC
LDHA	S	CTCCAAGCTGGTCATTATCACG
	A	AGTTCGGGCTGTATTTTACAACA
PDK1	S	TCCTGTCACCAGCCAGAATG
	A	CTTCCTTTGCCTTTTCCACC