SUPPLEMENTAL DATA

Rapamycin and Glucose-TOR Signaling in Plants

Yan Xiong and Jen Sheen

Supplementary Table 1. Primers used for constructs.

Name	Forward primer	Reverse primer
S6K1	ATGGTTTCCTCTCAGCGTCC	CAAAGTAGTTGTGGACTGGTGA
S6K2	ATGGTTTCTTCTCAGTGTTCTGTTG	CAAGTTGGATGTGGTCCGAT
FKP12	ATGGGAGTGCAGGTGGAA	TTCCAGTTTTAGAAGCTCCACAT
FKP15.1	ATGATGAGCTCTGCATCCG	AAGCTCATTCTTTGATTTCGCT
FKB15.2	ATGGCGAGCAAGATGAGTCT	TAGCTCGTCATTTCCATATCCC
FKP20.1	ATGGGTGATGCAATCGATTT	TTTAGCTTTGCCTTTGCCTTT
<i>FKP42</i>	ATGGATGAATCTCTGGAGCATC	ATCTGCTTTAACTCTGTGGCG
FKP62	ATGGATGCTAATTTCGAGATGC	AGACCICAGGIGCICATIGC
FKP65	ATGGAAGACGATTTCGACACG	TGCCTTGGTGTCAATACTCATC
FKP72	ATGGCGGTAGGCGATCAG	TGTAAATTTGGCGCTCACAA

Α																														
					_						- 0				1	FOR	2													
HEAT repea	its						E/	٩T	1	FR	в	Ki	nas	e 🛛 F.	ATC	2														
	-	-0-	- (1)		-	_			H						II F	/sT	OR													
															-															
В																														
TOR_kinase	1	IS	SF	SR	QL	$\vee \mathbf{V}$	11	r s I	ΚQ	RF	PR	ΚL	Т	ΙH	G	۱D	GE	D١	ΥA	FL	LK	GH	1 E (L	RQ	D	ERV	/ M G	LF	50
HsTOR_kinase	1	IQ	SI	ΑP	SL	QV	11	r s i	ΚQ	RF	P R	ΚL	Τl	M	GS	3 N	G⊢	ΕF	V	FL	LK	Gŀ	I E I	L	RQ	D	ERV	/ M Q	≀L F	50
TOR kinase	51	GL	VN	ті	I E	N S	R	(T)		ĸ		S I	O F	۶Y	S V	/ 1	ΡI	SF	N	S G		GW	/ / 6		сп	т	HE		RE	100
HsTOR_kinase	51	GL	V N	τĽ	LA	ND	P 1	s	LR	K		s i	QI	λΥ	AV	/ i	PL	S	N	SG	Ľi	GW	/ / /	H	СD	ті	НА		RD	100
					_	_				_	_	_			_	_	_					_						_		
TOR_kinase	101	HR	DA	R K	11	LN	Q	N	КН	ML	S	FA	PI	YC	DN	I L	P L	IA	K	V E	VF	EY	AL	E	NT	E	G N C)LS	RV	150
HSTOR_kinase	101	YR	EK	K K	I L	LN			RI	ML	R	MA	PI	D Y C	D			M C	א <u>א</u> ג	V E	VF	E	Α \	N	ΝΤ	A	GDL) L A	ΚL	150
TOR kinase	151	LW	LK	S R	s s	ΕV	W	EI	RR	TN	١Y	T R	SΙ	_ A '	VN	/ISI	мv	GY	1	LG	LG	DR	L H F	'S	NL	ML	HE	YS	GK	200
<i>H</i> sTOR_kinase	151	LW	LK	S P	s s	ΕV	W	D	RR	ΤN	١Y	ΤR	sι	- A '	V N	/ISI	мv	GΥ	11	LG	LG	DR	L H F	° S	NL	ML	DF	t L S	GK	200
TOR kinase	201	1.1	ні	DE	GР	CE	ΕA	9	MINI	RF	= K	FP	FK	C \/	PF	= P	ιт	RM	/ I	/ K	Δ Μ	EV	50	2 1	FG	NE	P	тс	EN	250
HsTOR_kinase	201	ΪĽ	нι	DF	GD	CF	ΕV		мΤ	RE	ΞK	FΡ	Eł	λ,	ΡF	R	ĽΤ	RN		ΤN	AM	EV	тс	L.	DG	NY	R I	тc	HT	250
		-																												
TOR_kinase	251	VM			TN	KD	SV	/ M /	A M	ME	A	FV		DP		N\	NR	L :	279											
nsi on_killase	251	VM		LK	ΕH	N D	21		A V		: A	ΓV	Υ	יאנ		. NI	NR	L	219											

Figure S1. TOR PKs are highly conserved in human and *Arabidopsis*. A, Domain organization of human and *Arabidopsis* TOR PKs. HEAT repeats: <u>Huntingtin, Elongation</u> factor 3, subunit of protein phosphatase $2\underline{A}$ and <u>T</u>OR1; FAT: FRAP, ATM and TRRAP domain; FRB: FKP12-rapamycin binding domain. FATC: Carboxy-terminal FAT domain. B, Alignment of the catalytic kinase domain of human and *Arabidopsis* TOR proteins.



Figure S2. S6Ks are highly conserved in human and *Arabidopsis*. A, Domain organization of human and *Arabidopsis* S6 kinases. B, Alignment of human and *Arabidopsis* S6 kinases. Asterisk indicates the conserved TOR phosphorylation sites.



Figure S3. T455 phosphorylation of S6K2. A, T455 phosphorylation specificity of S6K2. FLAG-tagged WT or mutant S6K2 (T455A) was transiently expressed in protoplasts. Total proteins were analyzed by protein blot analysis using anti-p-T389 (P-T455) or anti-FLAG (S6K2) antibody. B, T455 phosphorylation is abolished in the *tor* mutant. WT or conditional *tor* mutant protoplasts expressing FLAG-tagged S6K2 were treated without or with estradiol (10 μ M). Total proteins were analyzed by protein blot analysis probed with anti-TOR (TOR), anti-p-T389 (P-T455) or anti-FLAG (S6K2) antibody. Tubulin (TUB) was used as a loading control. C, T455 phosphorylation of S6K2 is inhibited by rapamycin (Rap).



Figure S4. Growth of *Arabidopsis* **seedlings relies on glucose.** WT and estradiol-inducible *tor* mutants were grown in liquid MS medium without any sugar or with 30 mM glucose for 9 days. Scale bar: 5 mm.



Figure S5. FKP12 is required for inhibition of seedlings growth by rapamycin. A, The inhibition effect of rapamycin on seedlings growth is relieved in *fkp12* mutants. The average root length was measured after 9 days of rapamycin (Rap, 10 μ M) treatment. Results represent means \pm SD (n=3). * indicates a significant difference with p<0.05 when compared with data from wild type (WT) treated with rapamycin, based on the results of an unpaired Student's t test. B, Overexpression of *Arabidopsis* or human FKP12 enhances the rapamycin inhibition on seedlings growth. The average root length was measured after 9 days of rapamycin (Rap) treatment. Results represent means \pm SD (n=3). * indicates a significant difference with p<0.05 when compared with data from well to seedlings growth. The average root length was measured after 9 days of rapamycin (Rap) treatment. Results represent means \pm SD (n=3). * indicates a significant difference with p<0.05 when compared with data from wild type (WT) untreated with rapamycin, based on the results of an unpaired Student's t test.

Α	FKP12 <i>H</i> sFKP12	1 1	M G V M G V	E K Q V	QV ET	IR IS	P G P G	ng. Dgr	- Pł rti	K P A F P K	R P C	G Q T G Q T	V T C V	V F V F	ICT IYT	G G A	= G k /I L E		GD G-	L S 	Q K K K	FW FD	S 3	Г К с 8 R с	R -	0 K P N K P	49 46
	FKP12 <i>H</i> sFKP12	50 47	FSF FKF	Q I M L	G K G K	G A Q E	V I I V I I * *	K GV R GV	V D I V E I	E G V E G V	/ I 0 / A 0	Э М С 2 М S		E V Q F	(A F R A K	R L F K L 7		S I P I	DY DY	AY AY *	G A G A	G G T G	F F H F	• A \\ • G	/ G - *	QPN PPH	99 95
	FKP12 <i>H</i> sFKP12	100 96	SVL AtL	D F V F	E I DV	EV EL	LSN LKI	VQ LE	112 108																		
в																											
	FRB <i>H</i> sFRB	1 1	ELI ELI	R V R V	A I A I	LW LW	H E I H E I	MW H MW H	1 E / 1 E (ALE GLE	EEA	SR SR *	LY LY *	F G F G *	SEF SEF	Η Ν Ι R Ν ∨	IE K	ЭМ ЭМ	L K F E	V L V L	E P E P	L H L H	D N A N	AL D Am e	E G R G	VKK PQ-	50 49
	FRB <i>Hs</i> FRB	51 50	D S T 	T I T L	Q E K E	R A T S	F I F N	E A) Q A)	(R (G	H E L R D L	K E M E	EA H EA C	H E (2 EV	C C C / C F	CNY RKY	(K) (M)	T (S (K N	D A V K	E L D L	TQ TQ *	AW AW *	D I D I *	YY YY YY	H V H V	F K R F R R *	100 96
	FRB <i>H</i> sFRB	101 97	IDK ISK	Q 1 Q 1	04 00																						

Figure. S6. FKP12s and TOR-FRB domains are highly conserved in human and *Arabidopsis.* Alignment of FKP12 proteins (A) and TOR-FRB domain (B) between human and *Arabidopsis.* Asterisks indicate conserved residues of human FKP12 and FRB that interact with rapamycin (1).



Figure S7. Root hairs are not affected in the *gin2* **mutant.** Ler and *gin2* were germinated with or without glucose (30 mM) for 4 days. Scale bar: 200 µm.

REFERENCES

1. Choi, J., Chen, J., Schreiber, S.L., and Clardy, J. (1996) Science 273, 239-242