## SUPPORTING INFORMATION

eIF4E1 regulates *Arabidopsis* embryo development and root growth by interacting with RopGEF7

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Figure S1. No GFP background signal in wild type embryos

No GFP fluorescence can be seen in 16-cell (A), globular (B), triangle (C), and heart (D) embryo stages of the wild type. The outlines of the embryos were stained by FM4-64 in red in image A to G, respectively. Scale bars: (A) 10  $\mu$ m; (B-D) 50  $\mu$ m.



Figure S2. Expression profiles of *eIF4E1*<sub>pro</sub>:GUS in Arabidopsis embryos
(A) Early globular, (B) globular, (C) early heart, (D) heart and (E) mature embryo stages. Scale bars: (A-C) 20 μm; (D, E) 50 μm.



## Figure S3. Subcellular localization of YFP-eIF4E1 fusion protein in the roots of four-day-old *eIF4E1*<sub>pro</sub>:YFP-eIF4E1 transgenic plants

YFP-eIF4E1 is predominantly localized at cytoplasm. (A) Bright field; (B) GFP; (C) Merged. Scale bars: 20 µm.



Figure S4. Knock-out or knock-down mutants of eIF4E1

(A) Structure of *eIF4E1*. Black and white boxes indicate exons and UTRs, respectively. The introns were displayed as black thin lines between exons. Two mutant lines, the one (*eif4e1-1*, CS6552) is single base replacement mutant (G to A) at 297 bp from the start code in the first exon resulting in tryptophan (Trp, TGG) to stop code (TGA), the other one (*eif4e1-2*, SALK\_145583C) harboring a T-DNA insertion in the first intron. The primer pairs P1/P2, and P3/P4 were designed for identifying the mutations. (B-C) Relative mRNA expression levels of *eif4e1-1* and *eif4e1-2* compared to wild type. The primer pair P5/P6 was designed for real-time PCR. Transcription levels were normalized to *ACTIN 2* (Fig. S1B) or *TUBULIN 2* (Fig. S1C), respectively. Data were presented as mean values of three biological repeats with SD.





(A) Comparison of RM and elongation region of six-day-old seedlings among wild type, *eif4e1-1*, *eif4e1-2*, and *eif4e1-1/eIF4E1<sub>pro</sub>:YFP-eIF4E1*. In order to show the meristem zone and entire elongation zone, Figure S5A is generated by three to four field photographs (using the Photoshop6 software overlap function). White lines in the middle of root tips indicate the length of RM. The region between two white arrowheads indicates the elongation zone. (B) The length of elongation zone. (C) Cell number of elongation zone. (D) Cell size of elongation zone. Data were presented as mean values with SD, n>30. The asterisks indicate significant difference by Student's *t* test (\*P<0.05, \*\*P<0.01, \*\*\*\*P<0.0001). Scale bars: 50 µm.



Figure S6. The relative expression levels of *PIN3* and *PIN7* in *eif4e1* mutants and wild type

Transcription levels were normalized to *ACTIN 2*. Data were presented as mean values of three biological repeats with SD.



Figure S7. *eIF4E1* mutation does not affect the accumulation of PIN1-GFP, PIN2-GFP and AUX1-YFP

(A-C) *PIN1*<sub>pro</sub>:*PIN1-GFP* is expressed in roots of four-day-old wild type (A), eif4e1-1 L1 (B) and eif4e1-1 L2 (C); (D-F) *PIN2*<sub>pro</sub>:*PIN2-GFP* is expressed in roots of four-day-old wild type (D), eif4e1-1 L1 (E) and eif4e1-1 L2 (F); and (G-I) *AUX1*<sub>pro</sub>:*AUX1-YFP* is expressed in roots of four-day-old wild type (G), eif4e1-1 L1 (H) and eif4e1-1 L2 (I). L1 and L2 indicate two individual lines. Scale bars: 20 μm.



Figure S8. CHX treatment does not affect the abundance of PIN1-GFP, PIN2 -GFPand AUX1-YFP fusion proteins

(A) Four-day-old seedlings of  $PIN1_{pro}$ : PIN1-GFP,  $PIN2_{pro}$ : PIN2-GFP and  $AUX1_{pro}$ : AUX1-YFP were transferred onto half-strength MS medium with or without 50 nM CHX for another three-day growth. (B) The relative root elongation of seedlings in the experiments of Figure S8A. Data were presented as mean values of three biological repeats with SD. (C-H) CHX treatment does not affect the abundance of PIN1, PIN2 and AUX1 proteins in roots of seedlings in the experiments of Figure S8A. (C, D)  $PIN1_{pro}$ : PIN1-GFP were treated with (D) or without (C) CHX. (E, F)  $PIN2_{pro}$ : PIN2-GFP were treated with (F) or without (E) CHX. (G-H) AUX1\_{pro}: AUX1-YFP were treated with (H) or without (G) CHX. Scale bars: (A) 1 cm; (C-H) 50  $\mu$ m.

Table S1. List of primers used in this study

Experiment	Primer name	Sequence		
Vector	eIF4E1p-GUS-F	AAGCTTTCTTTCTTTCTTTCCTCCTCTTTT		
construction	eIF4E1p-GUS-R	GGATCCTGTTTCTCCGAACTGCTTCTCTT		
	YFP-F	AAGCTTACAACTGCAGATGGTGAGCAAGGG		
	YFP-R	CCCGGGAAAAGGATCCTCTCTTGTACAGCTCGTC		
	eIF4E1 <sub>Pro</sub> -F	AAGCTTAGGGAAGGTTATTGCGTAGCAAGA		
	eIF4E1 <sub>Pro</sub> -R	CTGCAGTGTTTCTCCGAACTGCTTCTCTTTC		
	eIF4E1 CDS-F	GTCGACATGGCGGTAGAAGACACTCCCA		
	eIF4E1 CDS-R	CCCGGGTCAAGCGGTGTAAGCGTTCTTTG		
	eIF(iso)4E CDS-F	CATATGATGGCGACCGATGATGTGAAC		
	eIF(iso)4E CDS-R	GAATTCTCAGACAGTGAACCGGCTTCTT		
	RopGEF7-F	GAATTCATGGATGGTTCGTCGGAAAA		
	RopGEF7-R	GTCGACTCAAATCCCAGGATCAAGGTTC		
	GEF7-PRO-F	GAATTCAAGAAGATTCGTTCGGATTCA		
	GEF7-PRO-R	GTATACTCAGTTGTTGCCTAATGTTGTAGGAA		
	GEF7-∆N-F	GAATTCAAGAAGATTCGTTCGGATTCAAGA		
	GEF7-∆N-R	GTCGACTCAAATCCCAGGATCAAGGTTC		
	GEF7-C-F	ATCTCAGAGGAGGACCTG <u>CATATG</u> GGGAATGACG		
		CTCCTAAGAG		
	GEF7-C-R	GTCGACGGATCCCCGG <u>GAATTC</u> TCAAATCCCAGG		
		ATCAAGGTTC		
	GEF1-CDS-F	GAATTCATGGGGAGCTTATCTTCTGAGGA		
	GEF1-CDS-R	GTCGACATCTCTTTCCGGCGTCACTCCC		
	GEF1-PRO-F	GGAATTCCATATGGCAGATGTGGAGATGATGAAG		
		GAGA		
	GEF1-PRO-R	CGGGATTCGGTTGCTTTGTTAAGTCGTCCACGTA		
		G		
	GEF4-CDS-F	CTGATCTCAGAGGAGGACCTG <u>CATATG</u> ATGGAGA		
		GTTCTTCGAATTCCGACC		
	GEF4-CDS-R	CAGGTCGACGGATCCCCGG <u>GAATTC</u> CTAATCATC		
		TCTGTTTCTCACTGTTCTG		
	GEF4-PRO-F	CTGATCTCAGAGGAGGACCTG <u>CATATG</u> GCAGAGC		
		TAGAGATGATGAGGGAAA		
	GEF4-PRO-R	CAGGTCGACGGATCCCCGG <u>GAATTC</u> GTTTCTCAC		
		TGTTCTGTCGACGT		
	GEF6-CDS-F	GAATTCATGGAGGATAATAGCTGTATCGGGT		
	GEF6-CDS-R	GTCGACACCCCGGAGATAATTGGCCAATGCT		
	GEF6-PRO-F	ATGGCCATGGAGGCC <u>GAATTC</u> TCAGAGATTGAGT		
		TGTTGAAAGAGA		
	GEF6-PRO-R	TCGACGGATCCCCGG <u>GAATTC</u> ATCTTTGCTGATG		

		TCATCCATGAAC		
	35S-mCherry-GEF7-F	AGCTTCGAATTCTGCAGTCGACATGGATGGTTCG		
		TCGGAAAATT		
	35S-mCherry-GEF7-R	CGGACTCTAGATCAGGT <u>GGATCC</u> TCAAATCCCAG		
		GATCAAGGTTCG		
	35S-eIF4E1-eGFP-F	AGAACACGGGGGGAC <u>TCTAGA</u> ATGGCGGTAGAAG		
		ACACTCC		
	35S-eIF4E1-eGFP-R	GCCCTTGCTCACCAT <u>TCTAGA</u> AGCGGTGTAAGCG		
		TTCTTTG		
T-DNA	<i>eif4e1-1-</i> F	CGTTTTCAACTGTTGAGGAATTCTG		
mutant	eif4e1-1-R	TCTAATCCCCCAATAAAGAACATAA		
identification	<i>eif4e1-2-</i> F	TTCCATTGTTTTCCAATGCTC		
	<i>eif4e1-2-</i> R	GAAACAAACCTCTTGGGGAAG		
	LBb1.3	ATTTTGCCGATTTCGGAAC		
Real-time	<i>eIF4E1</i> qPCR-F (P5)	GAACGCTTACACCGCTTGAAA		
PCR	eIF4E1 qPCR-R (P6)	TTCGTGAACAATCAACACTAGCAA		
	qPIN3_F	AAGGAATTGTGCCCTTTGTG		
	qPIN3_R	TCGGAAGCGCTATAAGCATT		
	qPIN7_F	CGGGGAAGAAGAGTCGGAGAGG		
	qPIN7_R	GCAACAAGAGCCCAAATGAGACCA		
	ACTIN 2-F	ATGGCTGAGGCTGATGATATTCAAC		
	ACTIN 2-R	TACAAGGAGAGAACAGCTTGGATG		
	TUBULIN 2-F	ACAAACACAGAGAGGAGTGAGCA		
	TUBULIN 2-R	ACGCATCTTCGGTTGGATGAGTGA		

Genotyp	8-cel	globula	transitio	hear	Defect/	Percenta
e	1	r	n	t	Total	ge (%)
WT	1/31	0/66	0/12	1/58	2/167	1.20%
eif4e1-1	6/70	15/99	1/21	3/62	25/252	9.92%
eif4e1-2	5/67	13/118	1/23	4/51	23/259	8.88%

 Table S2. Quantitative analysis of embryonic phenotype of eif4e1-1 and eif4e1-2

 Table S3. Seedling defect rate analysis of eif4e1-1 and eif4e1-2

Genotype	Cotyledon defect number / total	Defect rate (%)
WT	1/162	0.62
eif4e1-1	13/168	7.74
eif4e1-2	10/146	6.8