

Fig S1. Lu et al.

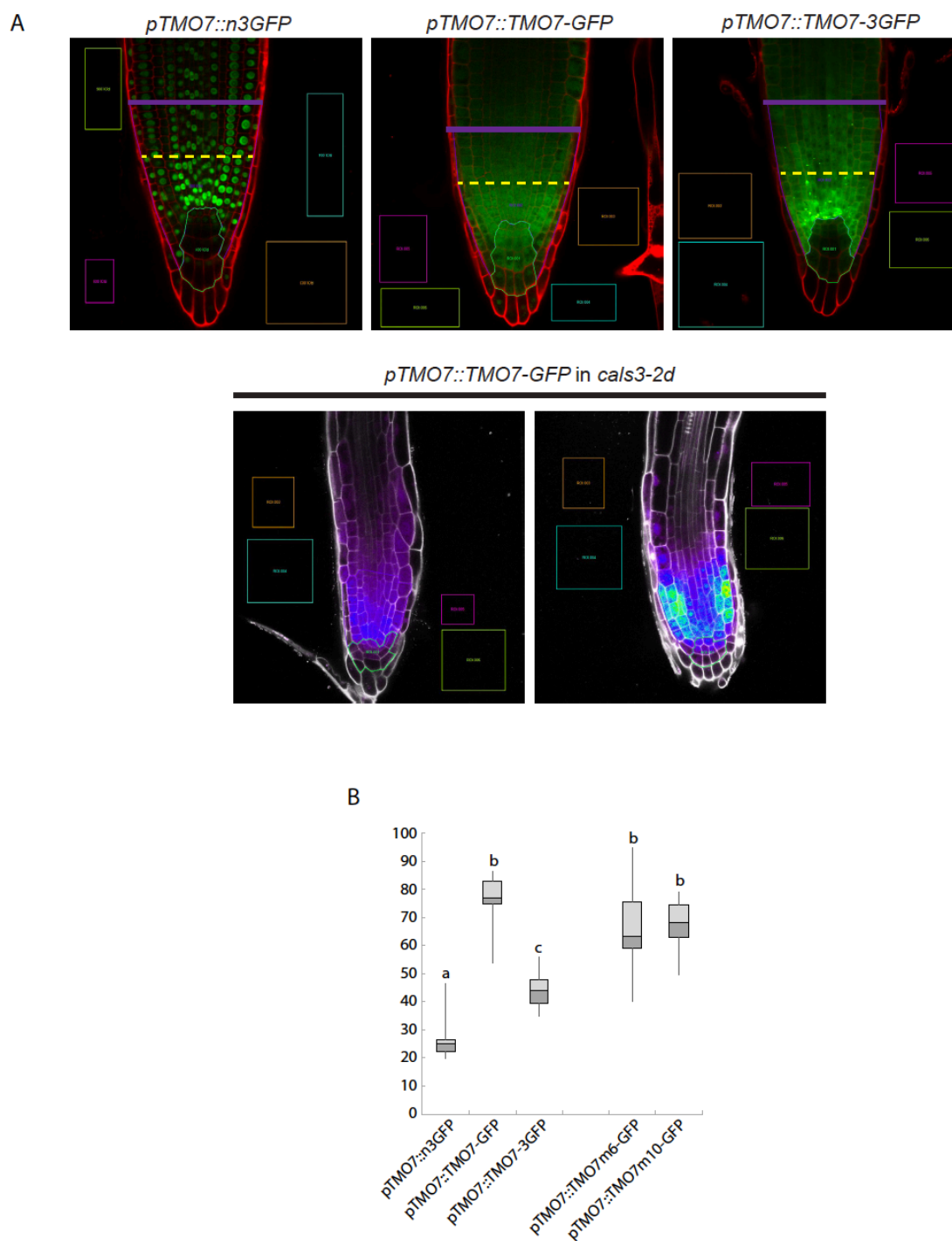


Fig S1. Sampling of Regions Of Interest (ROI) for statistical analysis. (A) Examples of confocal images analysed by the Leica Application Suite (LAS) program. The ROI1 (green region) was selected including the QC, and four layers of root cap cells. Regions outside the ROI1 up to the 12th cortex cell (indicated by the purple lines), besides the outer most root cap cells, were selected as the ROI2. Four other ROIs (ROI3-6) were selected to check the

background intensity. For selection in *cals3-2d*, the ROI1 and ROI2 were selected based on the morphology, the ROI1 includes 3 layers of cells from most distal region of the tip (excluding the most outer root cap cell), while ROI2 was selected based on the hypothetical cortex cells. (B) Intensity ratio of selected transgenic lines from Fig. 3 with narrower ROI2 region (indicated by the yellow dash lines). The results indicate that both TMO7_{m6}- and TMO7_{m10}-GFP have similar protein mobility to the TMO7-GFP.

Fig S2. Lu et al.

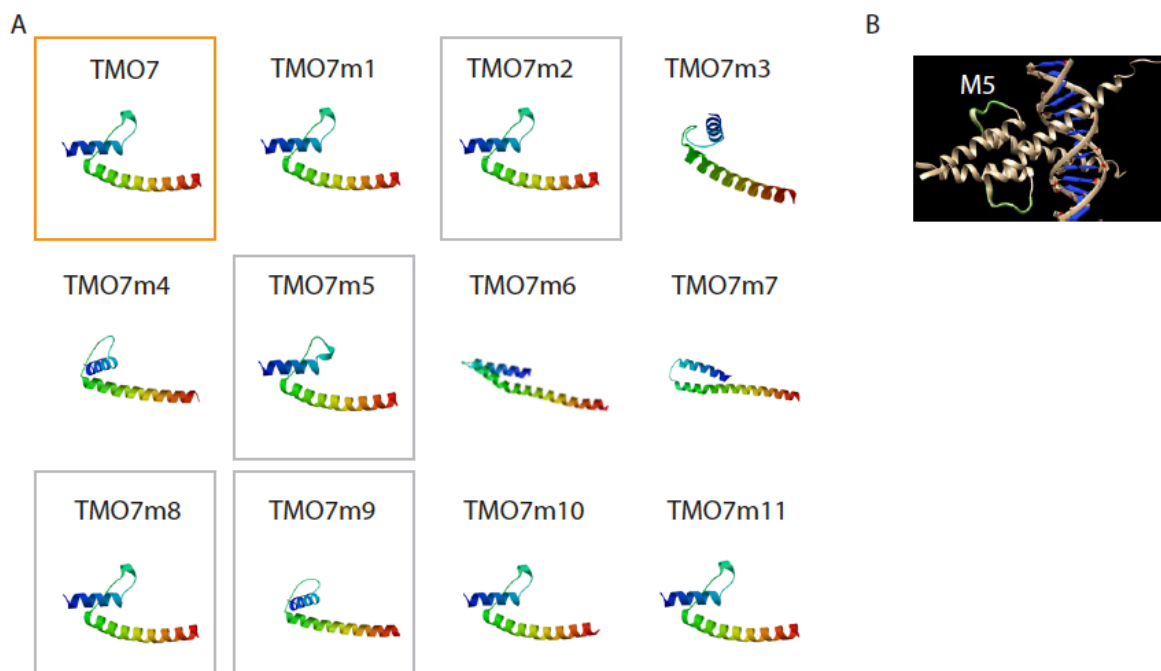


Fig S2. Structural homology models of TMO7 linker-scanning mutants. (A) The predicted structures of TMO7 and TMO7 mutants. The orange rectangle indicates the predicted TMO7 structure; Grey rectangles indicates the mutants affecting mobility. Note that only m9 has a minor predicted effect on structure while the remaining mobility mutants have the same predicted structure as TMO7. (B) The overlay of TMO7-M5 region on protein model of MyoD bHLH domain by chimera program (<https://www.cgl.ucsf.edu/chimera/>) with the PDB ID: 1MDY.

Fig S3 Lu et al.

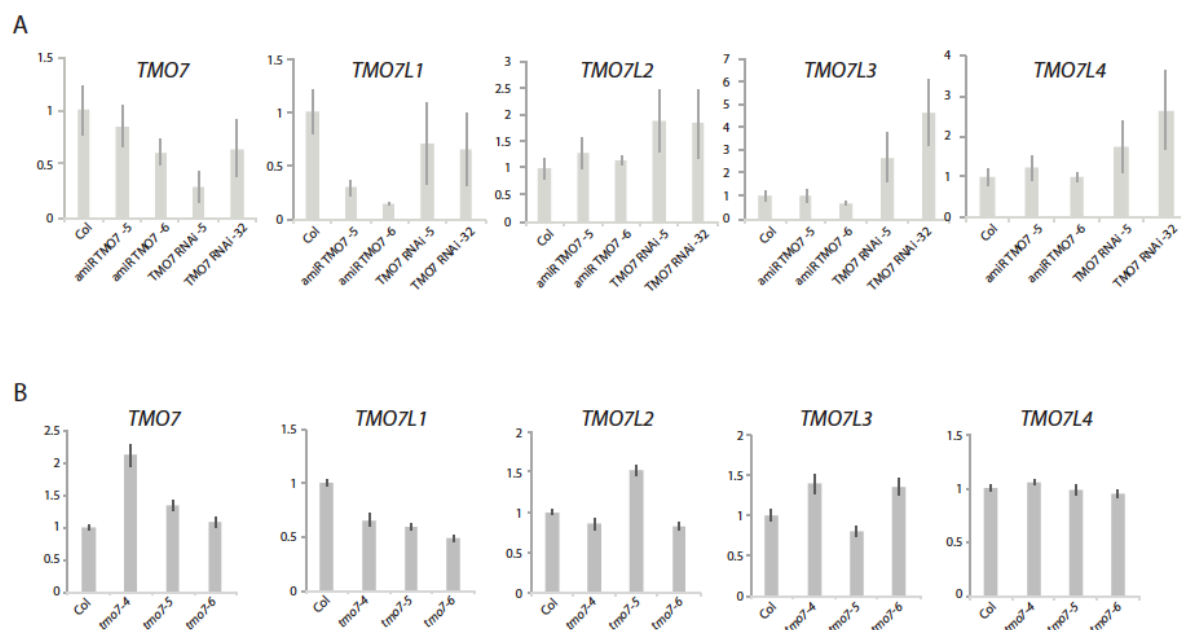


Fig. S3. Quantification of TMO7-Like genes in gene silencing lines and CRISPR/Cas9 generated *tmo7* mutants.

Expression level of *TMO7* family genes in (A) RNA suppression lines and (B) CRISPR/Cas9 *tmo7* lines by qRT-PCR analysis, relative expression level compared with endogenous control, *ACTIN2*. Note that in gene silencing lines, the *TMO7* family genes are highly interfered, while in CRISPR/Cas9 *tmo7* mutants, the gene expression is relatively stable. Quantified with three biological repeats and error bar indicates standard error of the mean.

Fig S4. Lu et al.

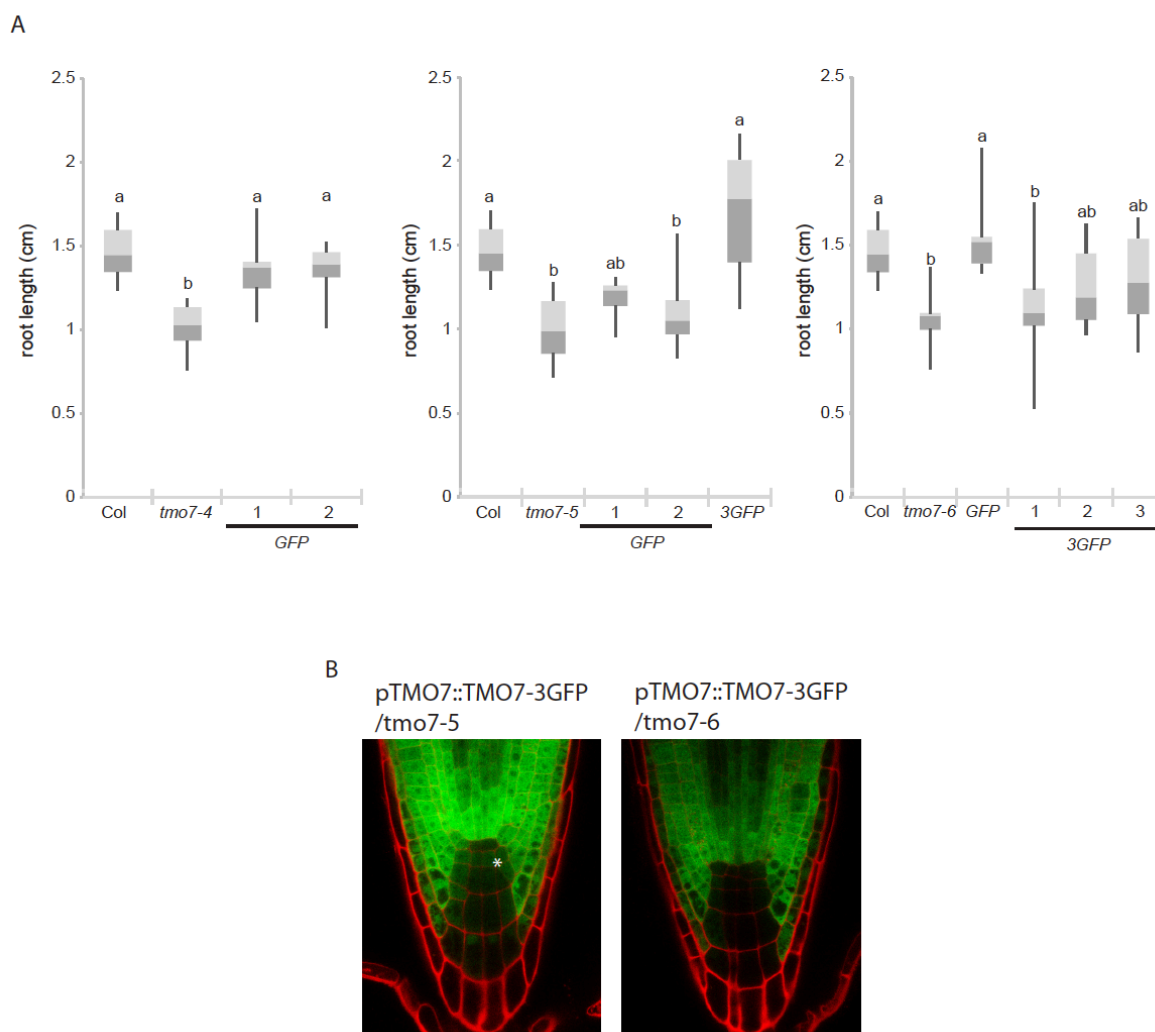


Fig S4. *tmo7* root complementation analysis and confocal images. (A) Box plot of 5-day-old seedling root length in different mutant background. Significant differences ($p < 0.05$), as determined by one-way ANOVA with Tukey's post-hoc analysis, are indicated by letters above bars. (B) Confocal images of pTMO7::TMO7-3GFP in *tmo7-5* (fully complement) and *tmo7-6* (no complementation) under the same confocal settings. Note that the expression level in the QC and columella cells in *tmo7-5* (indicated by the *) is higher than that in *tmo7-6*.

Table S1. List of oligo-nucleotides used in this research.

Name	Discription	Sequence	discription
KJ001	KJ LIC-TMO7 ^{pro} F	TAGTTGGAATAGGTTCTCGAGGTAGTTTCACTTT	forward primer for amplifying alenine linker mutant
KJ002	KJ TMO7 M1 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCCATTTTTTTGT AGAATATTG	reverse primer for 5' fragement of m1 mutant
KJ003	KJ TMO7 M2 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCTTGCCCTCGAA CGTGATCTTC	reverse primer for 5' fragement of m2 mutant
KJ004	KJ TMO7 M3 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCTTCTGAGATC CTTGAAGTTC	reverse primer for 5' fragement of m3 mutant
KJ005	KJ TMO7 M4 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCCTTGATAATC AGATCATTGA	reverse primer for 5' fragement of m4 mutant
KJ006	KJ TMO7 M5 N-term R	AGCTGCGGCCGCGGCAGCCGCCCTGAGCTCAGGAAGA AGCT	reverse primer for 5' fragement of m5 mutant
KJ007	KJ TMO7 M6 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCTGTCAATAT GTATCATGTA	reverse primer for 5' fragement of m6 mutant
KJ008	KJ TMO7 M7 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCATCTTGTAAC ACCCTGCTG	reverse primer for 5' fragement of m7 mutant
KJ009	KJ TMO7 M8 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCATGCAGATTC CGTATGTAGT	reverse primer for 5' fragement of m8 mutant
KJ010	KJ TMO7 M9 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCCCTCACTTA GATCATCAA	reverse primer for 5' fragement of m9 mutant
KJ011	KJ TMO7 M10 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCGTCTGAGTTT GCTAGTAACT	reverse primer for 5' fragement of m10 mutant
KJ012	KJ TMO7 M11 N-term R	GGCAGCTGCGGCCGCGGCAGCCGCTGCGCTTCTGATT AAAGCAGCTT	reverse primer for 5' fragement of m11 mutant
KJ013	KJ TMO7 M1 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCTCATCAGGAA CTTCAAGGAT	Forward primer for 3' fragment of m1 mutant
KJ014	KJ TMO7 M2 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCGATCAAATCA ATGATCTGATTA	Forward primer for 3' fragment of m2 mutant
KJ015	KJ TMO7 M3 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCTTGCAACAGC TTCTTCTGAG	Forward primer for 3' fragment of m3 mutant
KJ016	KJ TMO7 M4 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCAGGGACAGTC GTCGTCCGA	Forward primer for 3' fragment of m4 mutant
KJ017	KJ TMO7 M5 C-term F	GCGGCTGCCGCGGCCGCGAGCTGTATGCTGAATCTAACT AAGT	Forward primer for 3' fragment of m5 mutant
KJ018	KJ TMO7 M6 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCACGTGCAACT ACATACGGAA	Forward primer for 3' fragment of m6 mutant
KJ019	KJ TMO7 M7 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCAGAGAGGTTG ATGATCTAAG	Forward primer for 3' fragment of m7 mutant
KJ020	KJ TMO7 M8 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCCTATCTGAGTT ACTAGAAA	Forward primer for 3' fragment of m8 mutant
KJ021	KJ TMO7 M9 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCACTGCACAAG CTGCTTTAATCAGA	Forward primer for 3' fragment of m9 mutant
KJ022	KJ TMO7 M10 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCTACTTACCCA ATATCCTTATGA	Forward primer for 3' fragment of m10 mutant
KJ023	KJ TMO7 M11 C-term F	GCAGCGGCTGCCGCGGCCGCGAGCTGCCCTGATTATG CTGGATCCATGGT	Forward primer for 3' fragment of m11 mutant
KJ024	KJ GFP-LIC R	TTATGGAGTTGGGTTCCGAATTACTTGTACAGCTCGTCC A	Reverse primer for amplifying alenine linker mutant
KJ 276	KJ bHLH151 F	TAGTTGGAATGGGTTCCATGGGTGTAACATTAGAAGG	Forward primer for bHLH151
KJ 277	KJ bHLH151 R	AGCATAATCAGGAACATCATAAGGATAAACACAGTTA GTTTCGGTCA	Reverse primer for bHLH151
KJ 278	KJ GFP F	TATCCTTATGATGTTCTGATTATGCTATGGTGAGCAA GGGCGAGGA	Forward primer of GFP for bHLH151/138-GFP
KJ 279	KJ bHLH138 F	TAGTTGGAATGGGTTCCATGGAACGTTACACAAAAAA	Forward primer for bHLH138
KJ 280	KJ bHLH138 R	AGCATAATCAGGAACATCATAAGGATAAAAAGTGGTGT ACAAATCTAA	Reverse primer for bHLH138
KJ 301	KJ bHLH151M5 5'R	cttgctggaacgacgactgtccctaacaagctcttttagcgtct	Reverse primer for 5' fragment of M5 insertion

KJ 302	KJ bHLH151M5 3'F	agggacagctgctgctccgacaagtagtaggactctttagaca	Forward primer for 3' fragment of M5 insertion
KJ 305	KJ bHLH138M5 5'R	cttgctggaaacgacgactgtccctaatgaggtctttaaagtcga	Reverse primer for 5' fragment of M5 insertion
KJ 306	KJ bHLH138M5 3'F	agggacagctgctgctccgacaaggaagcatccattgttcaaga	Forward primer for 3' fragment of M5 insertion
KJ 311	KJ bHLH138M89 5'R	taactcagatagctcacttagatcatcaacctctctaactcgttgatgtaaacga	Reverse primer for 5' fragment of M8, M9 insertion
KJ 312	KJ bHLH138M89 3'F	ctaagtgagctatctgagttagctagcaaacactgacacaaagactagtttagcgagct	Forward primer for 3' fragment of M8, M9 insertion
KJ 313	KJ bHLH151M89 5'R	taactcagatagctcacttagatcatcaacctctcctcaagccaagataataatcg	Reverse primer for 5' fragment of M8, M9 insertion
KJ 314	KJ bHLH151M89 3'F	ctaagtgagctatctgagttagctagcaaacactgacagcaaatgaaagtgaaagtat	Forward primer for 3' fragment of M8, M9 insertion
KJ 240	KJ LIC-bHLH134 F	TAGTTGGAATGGGTTCCATGTCTTCTAGCAGAAGGTC	Forward primer for bHLH134-GFP
KJ 214	KJ bHLH134 R	TCCTCGCCCTTGCTCACCATTCATTAATCAAGCTCCTAA	Reverse primer for bHLH134-GFP
KJ 241	KJ LIC-bHLH136 F	TAGTTGGAATGGGTTCCATGTGCGAACAGAAGATCAAGG	Forward primer for bHLH136-GFP
KJ 216	KJ bHLH136 R	TCCTCGCCCTTGCTCACCATCATGAGTAGGCTTCTAATAA	Reverse primer for bHLH136-GFP
KJ 233	KJ LIC-bHLH161	TAGTTGGAATGGGTTCCATGGCGACGAACATCGGAAT	Forward primer for bHLH161-GFP
KJ145	KJ bHLH161 R	tcctcgcccttgctcaccatctgcataagcaaacctcgga	Reverse primer for bHLH161-GFP
KJ 242	KJ LIC-bHLH166 F	TAGTTGGAATGGGTTCCATGTCTAACAGAAGATCAAGG	Forward primer for bHLH166-GFP
KJ 219	KJ bHLH166 R	TCCTCGCCCTTGCTCACCATCATGAGTAAGCTTCTAATCA	Reverse primer for bHLH166-GFP
KJ 229	KJ LIC-NES-GFP R	TTATGGAGTTGGGTTTCATCAAGAGTAAGTCTTTCAAGAGGAGGAAGTTGAAGCTTGTACAGCTCGTCCATGC	Reverse primer for TMO7-GFP-NES
KJ 231	KJ LIC-NLS-TMO7 F	TAGTTGGAATGGGTTCCATGCCTAAGAAGAAGAGGAAAGGTTATGTCGGGAAGAAGATCACG	Forward primer for NLS-TMO7-GFP
KJ 281	KJ TMO7 S39A 5' R	cttgctggaaacgacgagcgtccct	Reverse primer for TMO7S39A 5' fragmen
KJ 282	KJ TMO7 S42A 5' R	cttgctggcaacgacgactgtccct	Reverse primer for TMO7S42A 5' fragment
KJ 283	KJ TMO7 S39A 3' F	agggacgctgctgctccgacaag	Forward primer for TMO7S39A 3' fragment
KJ 284	KJ TMO7 S42A 3' F	agggacagctgctgctccgacaag	Forward primer for TMO7S42A 3' fragment
KJ 249	KJ TMO7 CRISPR amplify F	ATTTTTCATAAAACAAATAAT	Forward primer for amplifying TMO7 genomic fragment for genotyping
KJ 250	KJ TMO7 CRISPR amplify R	CCTCTCTATGCAGATTCCGT	Reverse primer for amplifying TMO7 genomic fragment for genotyping
KJ 251	KJ TMO7 CRISPR sequencing F	acataataacaaccgtcact	sequencing primer for genotyping CRISPR/Cas9 tmo7
KJ526	KJ qbHLH134 F	GAACAAGGAAGCCGATGACC	qPCR primer for bHLH134
KJ527	KJ qbHLH134 R	GCTCCTAATAACTGCGGCTTG	qPCR primer for bHLH134
KJ530	KJ qbHLH136 F	GCATCAGCCTCGAAAGTATTGC	qPCR primer for bHLH136
KJ531	KJ qbHLH136 R	AAACGCTCGCTCAGATTGTC	qPCR primer for bHLH136
KJ534	KJ qbHLH161 F	AAGGAACCTGAGCAAAGAAGTGG	qPCR primer for bHLH161
KJ535	KJ qbHLH161 R	TTCGGATTAGTGCAGCTTGAG	qPCR primer for bHLH161
KJ538	KJ qbHLH166 F	TGTCAGCATCAAAGGTAACAAGA	qPCR primer for bHLH166
KJ539	KJ qbHLH166 R	TCAAGAAGCTGCGACAAACG	qPCR primer for bHLH166
BR210	Q_TMO7_end_F	CAACTACATACGGAATCT	qPCR primer for TMO7
BR211	Q_TMO7_end_R	AAGATAGATAGGAATTATTGG	qPCR primer for TMO7
JP001	ACT2	CTCCATTGTTTGTTCATT	qPCR primer for ACT2
JP002	ACT2	TCAATTCGATCACTCAGA	qPCR primer for ACT2
U6-F-Slice		ttactagataactagctgctggccgc TCGTTGAACAACGGAAACTCG	Forward primer for U6 promoter amplification with adaptor site for SLICE

TMO7 -U6- sg-R		TGTCGGAACGACGACTGTCCAATCACTACTTCGACTC TAG	Reverse primer with TMO7 sgRNA site to amplify U6 promoter
TMO7 -guide- F		GGACAGTCGTCGTTCCGACAGTTTATAGAGCTAGAAAT AGC	Forward primer with TMO7 sgRNA site to amplify sgRNA scaffold
guide- R-Slice		gcttgagctctcccatatggtcgaccGAATTCGAGCTCGGTACCC	Reverse primer for sgRNA scaffold amplification with adaptor site for SLiCE

Table S2. Two of the predicted phosphorylation sites are located in M5 region.

http://www.dabi.temple.edu/disphos/				
DISPHOS Results				
Position	Residue	Score	Sequence	Yes/No
2	S	0.987	***MSGRRS	YES
6	S	0.984	SGRRSRSRQ	YES
8	S	0.94	RRRSRQSS	YES
11	S	0.813	RSRQSSGTS	YES
12	S	0.682	SRQSSGTSR	YES
14	T	0.02	QSSGTSRIS	
15	S	0.691	SSGTSRISE	YES
18	S	0.479	TSRISEDQI	
39	S	0.751	ELR DSRRSD	YES
42	S	0.64	DSRRSDKVS	YES
46	S	0.295	SDKVSAARV	
54	T	0.006	VLQDTCNYI	
57	Y	0.02	DTCNYIRNL	
69	S	0.341	VDDLSERLS	
73	S	0.411	SERLSELLA	
79	S	0.141	LLANSDTAQ	
81	T	0.042	ANSDTAQAA	
89	S	0.13	ALIRSLLTQ	
92	T	0.006	RSLLTQ***	

Table S3. *tmo7-4*, -5 and -6 have significantly shorter root compare to Col.

	Length		Length		Length		Length
Col	2.69	<i>tmo7-4</i>	1.814	<i>tmo7-5</i>	1.885	<i>tmo7-6</i>	2.046
	2.645		1.812		1.74		1.683
	2.398		1.773		1.56		1.679
	2.393		1.731		1.543		1.612
	2.358		1.71		1.531		1.5
	2.333		1.703		1.487		1.491
	2.311		1.663		1.465		1.481
	2.228		1.602		1.463		1.472
	2.164		1.599		1.431		1.472
	2.137		1.584		1.369		1.461
	2.114		1.581		1.358		1.438
	2.093		1.531		1.29		1.344
	2.065		1.469		1.244		1.279
	1.897		1.428		1.209		1.203
	1.848		1.397		1.207		1.165
	1.77		1.367		1.165		1.159
	1.753		1.355		1.136		1.155
			1.298		1.093		1.026
			1.244		1.033		0.888
			0.952				
p-value		<i>tmo7-4</i>	1.59408E-09	<i>tmo7-5</i>	2.24588E-11	<i>tmo7-6</i>	3.38143E-10

Table S4 pTMO7::TMO7-GFP rescued the *tmo7* embryo phenotype.

Line	mutant number	wild type number	Total number	%
Col	5	217	222	2.25
<i>tmo7-4</i>	15	163	178	8.43
pTMO7::TMO7-GFP T2-1	5	216	221	2.26
pTMO7::TMO7-GFP T2-2	2	141	143	1.40
<i>tmo7-5</i>	9	127	136	6.62
pTMO7::TMO7-GFP T2-1	6	267	273	2.20
pTMO7::TMO7-GFP T2-2	5	228	233	2.15
pTMO7::TMO7-3GFP T2-1	3	188	191	1.57
<i>tmo7-6</i>	9	198	207	4.35
pTMO7::TMO7-GFP T2-2	2	144	146	1.37
pTMO7::TMO7-3GFP T2-2	9	197	206	4.37
pTMO7::TMO7-3GFP T2-3	11	252	263	4.18
pTMO7::TMO7-3GFP T2-5	14	224	238	5.88

Table S5. p*TMO7*::TMO7-GFP did not rescue the *cald3-2d* hypophysis phenotype

Lines in <i>cals3-2d</i>	mutant number	wild type number	Total number	%
p <i>TMO7</i> ::TMO7-GFP T3-10-2	189	3	192	1.56
p <i>TMO7</i> ::TMO7-GFP T3-15-5	220	2	222	0.90
p <i>TMO7</i> ::TMO7-GFP T3-15-9	193	3	196	1.53
pDR5::GFP T3-3-4	190	2	192	1.04
pDR5::GFP T3-10-1	143	2	145	1.38
pDR5::GFP T3-11-7	167	2	169	1.18