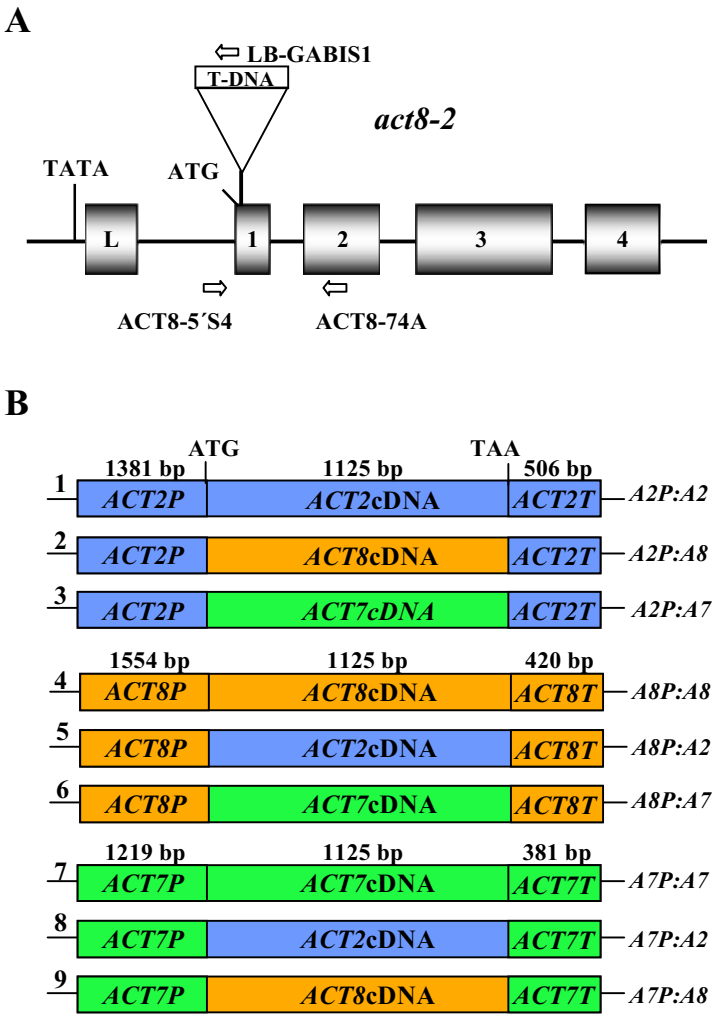
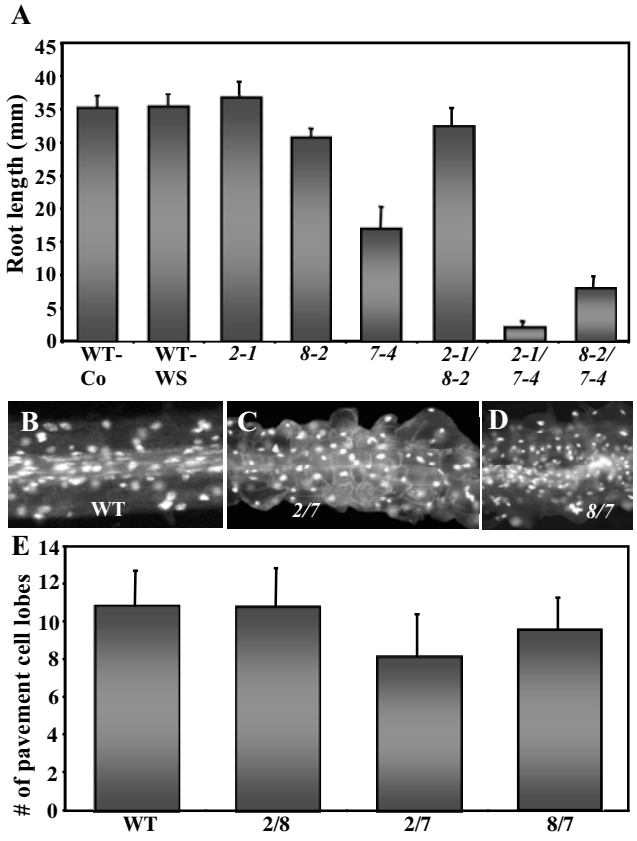
Supplemental Data. Kandasamy et al. (2009). A single vegetative actin isovariant overexpressed under the control of multiple regulatory sequences is sufficient for normal *Arabidopsis* development.



**Supplemental Figure 1**. *ACT8* Gene and Different Transgenic Constructs Used in Complementation/Suppression.

**(A)** Physical map of *act8-2* allele containing a T-DNA insertion at the ATG codon. The insertion deletes this codon along with 19 bases. The arrows indicate the location and orientation of primers that were used to distinguish native (ACT8-5′S4 and ACT8-74A) and T-DNA mutant (ACT8-5′S4 and LB-GABIS1) alleles. TATA box specifies the start of transcription. ATG = Start codon. L = Leader exon up-stream of exons 1, 2, 3 and 4.

**(B)** Actin overexpression constructs. Each of the nine different constructs are in three boxes (left box: promoter, 5′ untranslated region, leader exon to initiation codon ATG of exon #1; middle box: cDNA from different vegetative actin genes; right box: 3′ untranslated region and poly(A) sequences). TAA = Stop codon. *ACT2, ACT8* and *ACT7* regulatory and coding sequences are shown respectively in blue, orange and green.



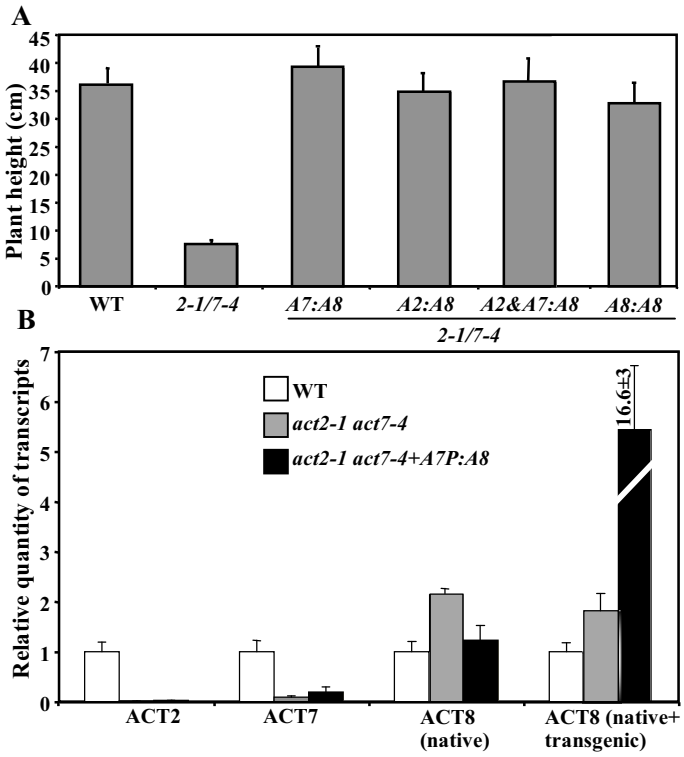

**Supplemental Figure 2.** Phenotypic Analysis of Vegetative Actin Single and Double Mutants.

**(A)** Root length of 10-day-old wild-type (Columbia [Co] and WS) and different mutant seedlings. N = 50. WT, wild-type; *2-1, act2-1; 8-2, act8-2; 7-4, act7-4; 2-1/8-2, act2-1 act8-2; 2-1/7-4, act2-1 act7-4; 8-2/7-4, act8-2 act7-4*.

**(B-D)** DAPI stained root portions of wild type, and *act2-1 act7-4* (*2/7*) and *act8-2 act7-4* (*8/7*) double mutants.

**(E)** Bar graph showing differences in the number of pavement cell lobes between wild-type and different double mutants. N = 30.

The bars in **(A)** and **(E)** represent standard deviation.




**Supplemental Figure 3.** Overexpression of ACT8 Restores Normal Plant Growth to *act2-1 act7-4* Double Mutants.

**(A)** Height of adult *act2-1 act7-4* (*2-1/7-4*) plants rescued by ACT8 expression under the control of *ACT7* (*A7:A8*), *ACT2* (*A2:A8*), *ACT8* (*A8:A8*), and *ACT2* and *ACT7* (*A2&A7:A8*) regulatory sequences. WT: wild-type. N = 20.

**(B)** qRT-PCR analysis of relative quantities of *ACT2*, *ACT7* and *ACT8* transcripts in wild-type, *act2-1 act7-4* double mutant and an *act2-1 act7-4* plant rescued with *A7P:A8*. The data represent average values of two technical replicates.

The error bars in **(A)** and **(B)** correspond to standard deviation.

**Supplemental Table 1.** Relative Transcript Levels in the *act2-1 act7-4* Double Mutant Plants Overexpressing ACT8 Under the Control of Various Actin Promoters.

| Plant | *ACT2* | *ACT7* | *ACT8* |
|---|---|---|---|
| Wild-type | 1.0 | 1.00 | 1.00 |
| *act2-1 act7-4* | 0 | 0.10 | 1.30 |
| KH-20 (A7P) | 0 | 0.03 | 17.0 |
| BK-5 (A2P) | 0 | 0.02 | 33.0 |
| BKH1-9 (A2&7P) | 0 | 0.05 | 38.0 |
| KH8-7 (A8P) | 0 | 0.03 | 4.00 |

**Supplemental Table 2.** Primers Used for Detection of Mutant Alleles and qRT-PCR.

*ACT2 & act2-1* PCR Primers:

ACT2-173S: 5´-CTTCCTCAATCTCATCTTCTT-3´
ACT2-AS : 5´-CATGACACCATGATGTCTTGGCCT-3´
ACT2-173S : 5´-GCTCAGGATCCGATTGTCGTTTCCCGCCTT-3´
Wild type: ACT2-173S & ACT2-AS; *act2-1*: ACT2-173S & ACT2-173S

*ACT2* Real-Time Primers:
ACT2-3´-RT-S: 5´-GATGAGGCAGGTCCAGGAATC-3´
ACT2-3´-RT-A: 5´-AACCCCAGCTTTTTAAGCCTTT-3´

*ACT7 & act7-4* PCR primers:

AAc7S: 5´-AGGATTCTTCTCGCTTCTGTCGATCTCTCGCT-3´
AAc7-I1A: 5´-AAATCATGATCAGTAGTCTTACACATGT-3´
JLB7804: 5´-TTGGTAATTACTCTTTCTTTTCCTCCATATT-3´
Wild-type: AAc7S & AAc7-I1A; *act7-4*: JLB7804 & AAc7-I1A

*ACT7* Real-Time Primers:
ACT7-RT-S: 5´-AGTCATAACCATCGGAGCTGAGA-3´
ACT7-RT-A: 5´-TGAGCGATGGCTGGAACA-3´

*ACT8 & act8-2 PCR* Primers:

ACT8-5´S4: 5´-TCGATCAAGATTCAGATCTTTATG-3´
ACT8-74A: 5´ -ACACCATGCTCAATAGGGTATTTCAAT-3´
LB-GABIS1: 5´ -CCCATTTGGACGTGAATGTAGACAC-3´
Wild-type: ACT8-5´S4 & ACT8-74A; *act8-2*: ACT8-5´S4 & LB-GABIS1

*ACT8* Real-Time Primers:

ACT8RTS2: 5´-AGCGGTTTTCCCCAGTGTT-3´
ACT8RTA2: 5´-TTCTGATTCATCCCAACCATGA-3´
ACT8-3´UTRrtS: 5´-TCCGGTTACAGCGTTTGGA-3´
ACT8-3´UTRrtA: 5´-GCGAGAGCGGGTTTTCAA-3´
Native: ACT8-3´UTRrtS & ACT8-3´UTRrtA
Native + transgenic: ACT8RTS2 & ACT8RTA2

*RP16A* Real-Time Primers:

RPS16A-real-S: 5´-TCTCGTTACCAGAAGAGTTACCG-3´
RPS16A-real-A: 3´ACCTTGAGATGAATCGAAATTGA-3´